

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>A01N 37/10, 55/02, C07C 229/00, C07D 211/78</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 98/39966</b> <b>(43) International Publication Date:</b> 17 September 1998 (17.09.98)
<b>(21) International Application Number:</b> PCT/US98/04763 <b>(22) International Filing Date:</b> 11 March 1998 (11.03.98) <b>(30) Priority Data:</b> 08/814,225 11 March 1997 (11.03.97) US <b>(71) Applicant:</b> BEACON LABORATORIES, L.L.C. [US/US]; Suite 303, Wayne Plaza 1, Wayne, NJ 07470 (US). <b>(72) Inventors:</b> NUDELMAN, Abraham; Miller Street 15, 76284 Rehovot (IL). REPHAELI, Ada; 16 Andrea Drive, North Caldwell, NJ 07006 (US). <b>(74) Agents:</b> WILSON, M., Lisa et al.; Kenyon & Kenyon, One Broadway, New York, NY 10004 (US).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> NITROGEN-CONTAINING OXYALKYLENE ESTERS AND USES THEREOF  <b>(57) Abstract</b>  This invention relates to compositions for and methods of treating, preventing or ameliorating cancer and other proliferative diseases as well as methods of inducing wound healing, treating cutaneous ulcers, treating gastrointestinal disorders, treating blood disorders such as anemias, immunomodulation, enhancing recombinant gene expression, treating insulin-dependent patients, treating cystic fibrosis patients, inhibiting telomerase activity, treating virus-associated tumors, especially EBV-associated tumors, modulating gene expression and particularly augmenting expression of tumor suppressor genes, inducing tolerance to antigens, treating, preventing or ameliorating protozoan infection or inhibiting histone deacetylase in cells. The compositions of the invention are to and the methods of the invention use nitrogen-containing oxyalkyl esters.		

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

NITROGEN-CONTAINING  
OXYALKYLENE ESTERS AND USES THEREOF

5     FIELD OF THE INVENTION

          This invention relates to compositions for and methods of treating, preventing or ameliorating cancer and other proliferative diseases as well as methods of inducing wound healing, treating cutaneous ulcers,  
10     treating gastrointestinal disorders, treating blood disorders such as anemias, immunomodulation, enhancing recombinant gene expression, treating insulin-dependent patients, treating cystic fibrosis patients, inhibiting telomerase activity, treating virus-associated tumors,  
15     especially EBV-associated tumors, modulating gene expression and particularly augmenting expression of tumor suppressor genes, inducing tolerance to antigens, treating, preventing or ameliorating protozoan infection or inhibiting histone deacetylase in cells. The  
20     compositions of the invention are to and the methods of the invention use nitrogen-containing oxyalkyl esters.

BACKGROUND OF THE INVENTION

          Butyric acid (BA) is a natural product. It is  
25     supplied to mammals from two main sources: 1) the diet, mainly from dairy fat, and 2) from the bacterial fermentation of unabsorbed carbohydrates in the colon, where it reaches mM concentrations (Cummings, Gut 22:763-779, 1982; Leder et al., Cell 5:319-322, 1975).

30     BA has been known for nearly the last three decades to be a potent differentiating and antiproliferative agent in a wide spectra of neoplastic cells in vitro (Prasad, Life Sci. 27:1351-1358, 1980). In cancer cells, BA has been reported to induce cellular and biochemical  
35     changes, e.g., in cell morphology, enzyme activity, receptor expression and cell-surface antigens (Nordenberg et al., Exp. Cell Res. 162:77-85, 1986; Nordenberg et al., Br. J. Cancer 56:493-497, 1987; and Fishman et al., J. Biol. Chem. 254:4342-4344, 1979).

Although BA or its sodium salt (sodium butyrate, SB) has been the subject of numerous studies, its mode of action is unclear. The most specific effect of butyric acid is inhibition of nuclear deacetylase(s), resulting in hyperacetylation of histones H3 and H4 (Riggs, et al., Nature 263:462-464, 1977). Increased histone acetylation, following treatment with BA has been correlated with changes in transcriptional activity and the differentiated state of cells (Thorne et al., Eur. J. Biochem. 193:701-713, 1990). BA also exerts other nuclear actions, including modifications in the extent of phosphorylation (Boffa et al., J. Biol. Chem. 256:9612-9621, 1981) and methylation (Haan et al., Cancer Res. 46:713-716, 1986). Other cellular organelles, e.g., cytoskeleton and membrane composition and function, have been shown to be affected by BA (Bourgeade et al., J. Interferon Res. 1:323-332, 1981). Modulations in the expression of oncogenes and suppressor genes by BA were demonstrated in several cell types. Toscani et al., reported alterations in c-myc, p53 thymidine kinase, c-fos and AP2 in 3T3 fibroblasts (Oncogene Res. 3:223-238, 1988). A decrease in the expression of c-myc and H-ras oncogenes in B16 melanoma and in c-myc in HL-60 promyelocytic leukemia was also reported (Prasad et al., Biochem. Cell Biol. 68:1250-1255, 1992; and Rabizadeh et al., FEBS Lett. 328:225-229, 1993).

BA has been reported to induce apoptosis, i.e., programmed cell death. SB has been shown to produce apoptosis *in vitro* in human colon carcinoma, leukemia and retinoblastoma cell lines (Bhatia et al., Cell Growth Diff. 6:937-944, 1995; Conway et al., Oncol. Res. 7:289-297, 1993; Hague et al.; Int. J. Cancer 60:400-406, 1995). Apoptosis is the physiological mechanism for the elimination of cells in a controlled and timely manner. Organisms maintain a delicate balance between cell proliferation and cell death, which when disrupted can

tip the balance between cancer, in the case of over accumulation of cells, and degenerative diseases, in the case of premature cell losses. Hence, inhibition of apoptosis can contribute to tumor growth and promote progression of neoplastic conditions.

The promising *in vitro* antitumor effects of BA and BA salts led to the initiation of clinical trials for the treatment of cancer patients with observed minimal or transient efficacy [Novogrodsky et al., Cancer 51:9-14, 1983; Rephaeli et al., Intl. J. Oncol. 4:1387-1391, 1994; Miller et al., Eur. J. Cancer Clin. Oncol. 23:1283-1287, 1987].

Clinical trials have been conducted for the treatment of  $\beta$ -globin disorders (e.g.,  $\beta$ -thalassemia and sickle-cell anemia) using BA salts. The BA salts elevated expression of fetal hemoglobin (HbF), normally repressed in adults, and favorably modified the disease symptoms in these patients (Stamatoyannopoulos et al., Ann. Rev. Med. 43:497-521, 1992). In this regard, arginine butyrate (AB) has been used in clinical trials with moderate efficacy (Perrine et al., N. Eng. J. Med. 328:81-86, 1993; Sher et al., N. Eng. J. Med. 332:1606-1610, 1995). The reported side effects of AB included hypokalemia, headache, nausea and vomiting in  $\beta$ -thalassemia and sickle-cell anemia patients.

Butyric acid derivatives with antitumor activity and immunomodulatory properties have been reported in U.S. Patent 5,200,553 and by Nudelman et al., 1992, J. Med. Chem. 35:687-694. The most active butyric acid prodrug reported in these references was pivaloyloxymethyl butyrate (AN-9). Similar compounds are reported for treating hemoglobinopathies (U.S. Patent No. 5,569,675).

BA and/or its analogues have also been reported to increase the expression of transfected DNA (Carstea et al., 1993, Biophys. Biochem. Res. Comm. 192:649; Cheng et al., 1995, Am. J. Physical 268:L615-L624) and to induce

tumor-restricted gene expression by adenovirus vectors  
(Tang et al., 1994, Cancer Gene Therapy 1:15-20).  
Tributyrin has been reported to enhance the expression of  
a reporter gene in primary and immortalized cell lines  
5 (Smith et al., 1995, Biotechniques 18:852-835).

However, BA and its salts are normally metabolized  
rapidly and have very short half-lives *in vivo*, thus the  
achievement and maintenance of effective plasma  
concentrations are problems associated with BA and BA  
10 salts, particularly for *in vivo* uses. BA and BA salts  
have required large doses to achieve even minimal  
therapeutic effects. Because of the high dosage, fluid  
overload and mild alkalosis may occur. Patients  
receiving BA emanate an unpleasant odor that is socially  
15 unacceptable.

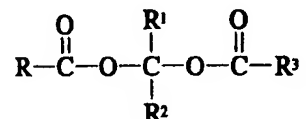
While BA salts have been shown to increase HbF  
expression, and appear to hold therapeutic promise with  
low toxicity in cancer patients, they nevertheless have  
shown low potency in *in vitro* assays and clinical trials.  
20 There remains a need to identify compounds as effective  
or more effective than BA or BA salts as differentiating  
or anti-proliferating agents for the treatment of  
cancers. Such compounds need to have higher potency than  
BA without the problems associated with BA (such as bad  
25 odor). This need can be addressed by therapeutic  
compounds that either deliver BA to cells in a longer  
acting form or which have similar activity as BA but a  
longer duration of effectiveness *in vivo*.

The compounds of this invention address these needs  
30 and are more potent than BA or BA salts for treating of  
cancers and other proliferative diseases, for treating  
gastrointestinal disorders, for wound healing, for  
treating blood disorders such as thalassemia, sickle cell  
anemia and other anemias, for modulating an immune  
35 response, for enhancing recombinant gene expression, for  
treating insulin-dependent patients, for treating cystic

fibrosis patients, for inhibiting telomerase activity, for treating virus-associated tumors, especially EBV-associated tumors, for modulating gene expression and particularly for augmenting expression of a tumor suppressor gene, for inducing tolerance to an antigen, for treating, preventing or ameliorating protozoan infection and for inhibiting histone deacetylase in cells. For example, one of the advantages of the compounds of the invention is increased water solubility of the free nitrogen-containing oxyalkylene esters compounds of the invention and their salts, and thus easier administration, especially for intravenous administration.

#### SUMMARY OF THE INVENTION

Accordingly, one embodiment of the present invention is directed to a method of treating preventing or ameliorating cancer and other proliferative disorders using compounds of Formula I represented by:



wherein

R is C<sub>2</sub> to C<sub>10</sub> alkyl, alkenyl or alkynyl, any of which can be optionally substituted with aryl, heteroaryl, halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro, trifluoromethyl, acylamino or carbamoyl;

R<sup>1</sup> and R<sup>2</sup> are independently H, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>2</sub>-C<sub>10</sub> alkenyl, or C<sub>2</sub>-C<sub>10</sub> alkynyl, any of which can be optionally substituted with halo, alkoxy, amino, trifluoromethyl, aryl or heteroaryl;

R<sup>3</sup> is

(1) a nitrogen-containing heterocycloalkyl, heterocycloalkenyl, heteroaryl group or heteroaralkyl group, wherein the heteroaryl group and the heteroaralkyl group can have up to two additional substituents;

(2) an aryl or aralkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, alkoxycarbonylamino, formamido, guanidino, ureido, sulfamyl, and alkylsulfonamido, wherein the aryl or aralkyl groups can have up to two additional substituents; or

(3) a C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl, C<sub>3</sub> to C<sub>10</sub> alkynyl or C<sub>3</sub> to C<sub>7</sub> cycloalkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, alkoxycarbonylamino, formamido, guanidino, ureido, sulfamyl, and alkylsulfonamido; and

pharmaceutically-acceptable salts thereof;

with the provisos that R is not substituted or unsubstituted n-propyl; and that when R is t-butyl and R<sup>1</sup> and R<sup>2</sup> are both hydrogen, then R<sup>3</sup> can not be 3-aminopropyl or 3-(t-butoxycarbonylamino)propyl. The above compounds are referred to herein as "Formula I compounds."

In addition the invention includes compounds for use in the above method and designated herein as Formula Ia compounds, which are defined the same as in Formula I except that R is substituted or unsubstituted n-propyl (where the substituents are the same as provided for R of



Formula I), and that when  $R^3$  is  $C_1 - C_{10}$  alkyl, then this moiety can not have an amino substituent.

In a preferred embodiment, the compounds used in the methods of this invention are those of Formula I or Ia  
5 wherein

R is  $C_3$  to  $C_6$  alkyl, alkenyl or alkynyl, any of which can be optionally substituted with aryl, heteroaryl, halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro,  
10 trifluoromethyl, acylamino or carbamoyl;

$R^1$  and  $R^2$  are independently H,  $C_1 - C_{10}$  alkyl,  $C_2 - C_{10}$  alkenyl, or  $C_2 - C_{10}$  alkynyl, any of which can be optionally substituted with halo, alkoxy, amino, trifluoromethyl,  
15 aryl or heteroaryl;

$R^3$  is

(1) a nitrogen-containing heterocycloalkyl, heteroaryl group or heteroaralkyl group, wherein the  
20 heteroaryl group and the heteroaralkyl group can have up to two additional substituents;

(2) an aryl or aralkyl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino,  
25 dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, and alkoxycarbonylamino, wherein the aryl or aralkyl groups can have up to one additional substituent; or

(3) a  $C_1$  to  $C_{10}$  alkyl,  $C_2$  to  $C_{10}$  alkenyl,  $C_3$  to  $C_{10}$  alkynyl or  $C_3$  to  $C_7$  cycloalkyl group having one  
30 nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino and alkoxycarbonylamino; and

35 pharmaceutically-acceptable salts thereof.

In a more preferred embodiment,  $R^2$  is H. Yet another preferred embodiment is directed to Formula Ia wherein

R is n-propyl;

5

$R^1$  is H or alkyl and  $R^2$  is H;

$R^3$  is

10

(1) a nitrogen-containing heteroaryl group, wherein the heteroaryl group can have one additional substituent;

15

(2) an aryl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, and wherein the aryl group can have up to two additional substituents; or

20

(3) a  $C_1$  to  $C_{10}$  alkyl group having one nitrogen-containing substituent selected from the group consisting of acylamino, arylcarbonylamino and alkoxycarbonylamino; and

pharmaceutically-acceptable salts thereof.

25

The preferred compounds used in the methods of this invention are

4-(butyramido)butyroyloxymethyl butyrate, (BuBuOMB);

4-(butyramido)benzoyloxymethyl butyrate, (BuBOMB);

4-aminobenzoyloxymethyl butyrate, (AmBOMB);

30

4-aminobenzoyloxymethyl butyrate hydrochloride, (AmBOMB•HCl);

1-(4-aminobenzoyloxy)ethyl butyrate;

3-pyridinecarboxymethyl butyrate, (POMB);

1-(3-pyridinecarboxy)ethyl butyrate; and

(N-methylpyridinium)-3-carboxymethyl butyrate chloride,

35

(MePOMB•Cl).

The compounds of Formula I and Ia are particularly useful for methods of treating, preventing or ameliorating the effects of cancer and other proliferative disorders by acting as anti-proliferative or differentiating agents in subjects afflicted with such anomalies. Such disorders include but are not limited to leukemias, such as acute promyelocytic leukemia, acute myeloid leukemia, and acute myelomonocytic leukemia; other myelodysplastic syndromes, multiple myeloma such as but not limited to breast carcinomas, cervical cancers, melanomas, colon cancers, nasopharyngeal carcinoma, non-Hodgkins lymphoma (NHL), Kaposi's sarcoma, ovarian cancers, pancreatic cancers, hepatocarcinomas, prostate cancers, squamous carcinomas, other dermatologic malignancies, teratocarcinomas, T-cell lymphomas, lung tumors, gliomas, neuroblastomas, peripheral neuroectodermal tumors, rhabdomyosarcomas, and prostate tumors and other solid tumors. It is also possible that compounds of Formula I and Ia have anti-proliferative effects on non-cancerous cells as well, and may be of use to treat benign tumors and other proliferative disorders such as psoriasis. Preferred is the method for treating or ameliorating leukemia, squamous cell carcinoma and neuroblastoma.

The invention is further directed to a method of treating blood disorders by administering a therapeutically-effective amount of a compound of Formula I or Ia to a patient.

The blood disorders treatable in accordance with the invention include, but are not limited to, thalassemias, sickle cell anemias, infectious anemias, aplastic anemias, hypoplastic and hypoproliferative anemias, sideroblastic anemias, myelophthisic anemias, antibody-mediated anemias, anemias due to chronic diseases and enzyme-deficiencies, and anemias due to blood loss, radiation therapy and chemotherapy. In this regard,

these methods can include increasing hemoglobin content in blood by administering a therapeutically-effective amount of a compound of Formula I or Ia to a subject.

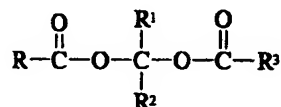
5 Another embodiment of the invention is directed to a method of modulating an immune response in a host by administering an amount of a compound of Formula I or Ia effective to modulate said immune response.

10 Modulation of the immune response includes enhancing cytokine secretion, inhibiting or delaying apoptosis in polymorphonuclear cells, enhancing polymorphonuclear cell function by augmenting hematopoietic growth factor secretion, inducing expression of cell surface antigens in tumor cells, and enhancing progenitor cell recovery after bone marrow transplantation.

15 Another embodiment of the present invention is directed to methods of treating, preventing or ameliorating cancer and other proliferative disorders by administering a therapeutically-effective amount of a compound of Formula II to a subject suffering from such disorders together with a pharmaceutical agent (e.g., a  
20 known antiproliferative, differentiating or oncostatic agent) to thereby enhance the action of these agents.

The compounds of Formula II are represented by the formula

25



wherein

30 R is C<sub>2</sub> to C<sub>10</sub> alkyl, alkenyl or alkynyl, any of which can be optionally substituted with aryl, heteroaryl, halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro, trifluoromethyl, acylamino or carbamoyl;

$R^1$  and  $R^2$  are independently H,  $C_1$ - $C_{10}$  alkyl,  $C_2$ - $C_{10}$  alkenyl, or  $C_2$ - $C_{10}$  alkynyl, any of which can be optionally substituted with halo, alkoxy, amino, trifluoromethyl, aryl or heteroaryl;

5

$R^3$  is

- (1) a nitrogen-containing heterocycloalkyl, heterocycloalkenyl, heteroaryl group or heteroaralkyl group, wherein the heteroaryl group and the heteroaralkyl group can have up to two additional substituents;
- (2) an aryl or aralkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, alkoxycarbonylamino, formamido, guanidino, ureido, sulfamyl, and alkylsulfonamido, wherein the aryl and aralkyl groups can have up to two additional substituents; or
- (3) a  $C_1$  to  $C_{10}$  alkyl,  $C_2$  to  $C_{10}$  alkenyl,  $C_3$  to  $C_{10}$  alkynyl or  $C_3$  to  $C_7$  cycloalkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, alkoxycarbonylamino, formamido, guanidino, ureido, sulfamyl, and alkylsulfonamido; and

10

15

20

25

30

pharmaceutically-acceptable salts thereof.

35

The compounds of Formula II are nearly the same as covered by the combination of both Formula I and Ia except that the compounds excluded by the provisos therein are included within the class of compounds represented by Formula II. In this regard, the preferred, more preferred and most preferred compounds

are the same as for the methods which use Formula I and Ia.

5 The pharmaceutical agents of the invention for the above method include, but are not limited to, cytokines, interleukins, anti-cancer agents, chemotherapeutic agents, antibodies, conjugated antibodies, immune  
10 stimulants, antibiotics, hormone antagonists, and growth stimulants. The compounds of the invention can be administered prior to, after or concurrently with any of the agents.

Yet another embodiment of the invention is directed to a method of ameliorating the effects of a cytotoxic agent which comprises administering a therapeutically-effective amount of a cytotoxic agent with a compound of  
15 Formula II to a mammalian patient for a time and in an amount to induce growth arrest of rapidly-proliferating epithelial cells of the patient and thereby protect those cells from the cytotoxic effects of the agent. The cytotoxic agent can be a chemotherapeutic agent, an  
20 anticancer agent, or radiation therapy. Rapidly proliferating epithelial cells are found in hair follicles, the gastrointestinal tract and the bladder, for example. Such cells include hair follicle cells, or intestinal crypt cells. Rapidly proliferating cells are  
25 also found in the bone marrow and include bone marrow stem cells. In accordance with the invention the cytotoxic agent and the compound of Formula II can be administered simultaneously, or the cytotoxic agent can be administered prior to or after the compound of the  
30 invention. Administration (simultaneously or separately) can be done systemically or topically as determined by the indication. In addition, when the cytotoxic agent is radiation therapy, the compounds of the invention can be administered to a cancer patient pre- or post-radiation  
35 therapy to treat or ameliorate the effects of cancer.

A still further embodiment of the invention is directed to a method of inducing wound healing, treating cutaneous ulcers or treating a gastrointestinal disorder by administering a therapeutically-effective amount of a compound of Formula II to a subject in need of such treatment. The cutaneous ulcers which can be treated in accordance with the methods of the invention include leg and decubitus ulcers, stasis ulcers, diabetic ulcers and atherosclerotic ulcers. With respect to wound healing, the compounds are useful in treating abrasions, incisions, burns, and other wounds. Gastrointestinal disorders treatable by the methods of the invention include colitis, inflammatory bowel disease, Crohn's disease and ulcerative colitis.

A further embodiment of the invention relates to a method of enhancing recombinant gene expression by treating a recombinant host cell containing an expression system for a mammalian gene product of interest with an expression-enhancing amount of a compound of Formula II, wherein said gene product is encoded by a butyric acid-responsive gene. The host cells can be mammalian cells, insect cells, yeast cells or bacterial cells and the correspondingly known expression systems for each of these host cells. The gene product can be any protein or peptide of interest, expression of which can be regulated or altered by butyric acid or a butyric acid salt. A butyric acid-responsive gene is a gene that has a promoter, enhancer element or other regulon that modulates expression of the gene under its control in response to butyric acid or a salt of butyric acid. For example, gene products contemplated for regulation in accordance with the invention include but are not limited to tumor suppressor genes (such as p53) and the  $\gamma$ -globin chain of fetal hemoglobin.

Yet a further embodiment of the invention is directed to a method of treating, preventing or

ameliorating symptoms in insulin-dependent patients by administering an amount of a compound of Formula II effective to enhance insulin expression.

5 Yet another embodiment of the invention relates to a method of treating, preventing or ameliorating symptoms in cystic fibrosis patients by administering an amount of a compound of Formula II effective to enhance chloride channel expression.

10 Still another method of the invention is directed to a method of inhibiting telomerase activity in cancer cells by administering a telomerase-inhibiting amount of a compound of Formula II to the cells, wherein the amount is effective to decrease the telomerase activity of the cells and thereby inhibit the malignant progression of  
15 the cells. This method can be applied to *in vivo* or *in vitro* cells.

Another embodiment of this invention is directed to a method of treating, preventing or ameliorating virus-associated tumors by pre-, post or co-administering a  
20 therapeutically-effective amount of a compound of Formula II with a therapeutically-effective amount of an antiviral agent. Antiviral agents contemplated for use in the invention include ganciclovir, acyclovir and famciclovir, and preferably ganciclovir. The virus-associated tumors which can be treated, prevented or  
25 ameliorated in accordance with the invention include, but are not limited to, EBV-associated malignancy, Kaposi's sarcoma, AIDS-related lymphoma, hepatitis B-associated malignancy or hepatitis C associated malignancy. EBV-associated malignancies include nasopharyngeal carcinoma  
30 and non-Hodgkins' lymphoma and are preferred embodiments of the invention.

Further still the invention provides a method of modulating gene expression by treating a host or host  
35 cells with an amount of a compound of Formula II effective to enhance, augment or repress the expression



of a gene of interest, preferably a butyric acid responsive gene. When expression of the gene of interest is to be enhanced or augmented, the gene can encode a gene product which is or acts as a repressor of another gene, a tumor suppressor, an inducer of apoptosis or an inducer of differentiation. When expression of the gene of interest is to be repressed, the gene can encode a gene product which is or acts as an oncogene or an inhibitor of apoptosis. For example, the bcl-2 gene encodes an inhibitor of apoptosis.

More particularly, the invention is directed to a method of augmenting gene expression, especially of a tumor suppressor gene, a butyric acid-responsive gene or a fetal hemoglobin gene, by treating a host or host cells with an expression-enhancing amount of a compound of Formula II. Preferably the host is a cancer patient. This method of the invention thus includes augmenting tumor suppressor gene expression in conjunction with *ex vivo* or *in vivo* gene therapy, i.e., the compound of the invention can be co-administered to the host during administration of gene therapy vectors or administration of the *ex vivo* transfected cells. Similarly, the compounds of the invention can be used to treat cells during the transfection step of *ex vivo* gene therapy. The hosts of the method therefore include cancer patients or other patients under going gene therapy. The host cells of the invention include hematopoietic cells such as stem cells and progenitor cells, e.g., or any other cell type used in *ex vivo* gene therapy.

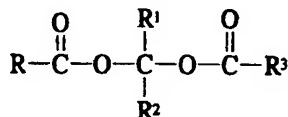
Yet another embodiment of the invention is directed to a method of inducing tolerance to an antigen which comprises administering a therapeutically-effective amount of compound of Formula II. Preferably the antigen is a self-antigen. For example, the self antigen can be associated with an autoimmune disease such as systemic lupus erythromatosis, rheumatoid arthritis, multiple

sclerosis or diabetes. Alternatively, tolerance can be induced to one or more antigens present on a transplanted organ or cells.

5 Yet further the invention is directed to a method for treating, preventing, or ameliorating protozoan infection in a subject which comprises administering to said subject an effective amount of a compound of Formula II. The protozoan infections treatable in accordance with the invention include, but are not limited to,  
10 malaria, cryptosporidiosis, toxoplasmosis, or coccidiosis.

Still further the invention is directed to a method of inhibiting histone deacetylase in cells which comprises administering an effective amount of a compound  
15 of Formula II to said cells.

The invention is also directed to compounds represented by the formula:



20 wherein

R is C<sub>2</sub> to C<sub>10</sub> alkyl, alkenyl or alkynyl, any of which can be optionally substituted with aryl, heteroaryl, halo, alkyl, alkoxy, hydroxy, amino, cyano,  
25 nitro, trifluoromethyl, acylamino or carbamoyl;

R<sup>1</sup> and R<sup>2</sup> are independently H, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>2</sub>-C<sub>10</sub> alkenyl, or C<sub>2</sub>-C<sub>10</sub> alkynyl, any of which can be optionally substituted with halo, alkoxy, amino, trifluoromethyl,  
30 aryl or heteroaryl;

R<sup>3</sup> is

(1) a nitrogen-containing heterocycloalkyl or heteroaryl group, wherein the heteroaryl group can have up to two additional substituents;

5 (2) an aryl or aralkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, and acylamino, wherein the aryl or aralkyl group can have up to two additional substituents; or

10 (3) a C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl, or C<sub>3</sub> to C<sub>7</sub> cycloalkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino and ureido; and

15 pharmaceutically-acceptable salts thereof;

20 with the provisos that R is not substituted or unsubstituted n-propyl; that when R is t-butyl and R<sup>1</sup> and R<sup>2</sup> are both hydrogen, then R<sup>3</sup> can not be 3-aminopropyl or 3-(t-butoxycarbonylamino)propyl; that when R is isopropyl and R<sup>1</sup> and R<sup>2</sup> are both hydrogen, then R<sup>3</sup> can not be 2-amino-6-chlorophenyl or 8-(3,7-dichloro-quinoline); and

25 that when R is isopropyl, R<sup>1</sup> is hydrogen, and R<sup>2</sup> is methyl, then R<sup>3</sup> can not be 4-(methylamino)cyclohexane.

The above compounds are referred to herein as "Formula III compounds."

30 In addition, the invention is directed to compounds designated as Formula IIIa which are the same as Formula I except that R is substituted or unsubstituted n-propyl (where the substituents are the same as provided for R of Formula III), and that when R<sup>3</sup> is C<sub>1</sub>-C<sub>10</sub> alkyl, then this group can not have an amino substituent.

35 In a preferred embodiment, the compounds of the invention are those of Formula III and IIIa wherein

R is C<sub>3</sub> to C<sub>6</sub> alkyl, alkenyl or alkynyl, any of which can be optionally substituted with aryl, heteroaryl, halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro, trifluoromethyl, acylamino or carbamoyl;

5

R<sup>1</sup> and R<sup>2</sup> are independently H, lower alkyl or lower alkenyl;

R<sup>3</sup> is

10

(1) a nitrogen-containing heterocycloalkyl or heteroaryl group, wherein the heteroaryl group can have up to two additional substituents;

15

(2) an aryl or aralkyl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, and alkoxycarbonylamino, wherein the aryl or aralkyl group can have up to one additional substituent; or

20

(3) a C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl, C<sub>3</sub> to C<sub>10</sub> alkynyl or C<sub>3</sub> to C<sub>7</sub> cycloalkyl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino and alkoxycarbonylamino; and

25

pharmaceutically-acceptable salts thereof.

In a more preferred embodiment, R<sup>2</sup> is H. Yet another preferred embodiment is directed to Formula IIIa wherein

30

R is n-propyl;

R<sup>1</sup> is H or alkyl and R<sup>2</sup> is H;

35

R<sup>3</sup> is

(1) a nitrogen-containing heteroaryl group, wherein said heteroaryl group can one additional substituent;

5 (2) an aryl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, and wherein the aryl group can have up to two additional substituents; or

10 (3) a C<sub>1</sub> to C<sub>10</sub> alkyl group having one nitrogen-containing substituent selected from the group consisting of acylamino, arylcarbonylamino and alkoxycarbonylamino.

The preferred compounds of the invention are  
15 BuBuOMB, BuBOMB, AmBOMB, AmBOMB•HCl, POMB, MePOMB•Cl, 1-(4-aminobenzoyloxy)ethyl butyrate and 1-(3-pyridinecarboxy)ethyl butyrate.

Another embodiment of the present invention is drawn to pharmaceutical compositions comprising a  
20 therapeutically effective amount of a compound of Formula III or IIIa and a pharmaceutically effective carrier or diluent.

A further embodiment of the present invention is directed to pharmaceutical compositions comprising a  
25 therapeutically effective amount of a combination of a compound of Formula III or IIIa with other anti-cancer or antineoplastic agents together with a pharmaceutically effective carrier or diluent.

30 BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a graphic illustration showing the *in vitro* inhibition of cellular growth (clonogenicity) by AB, AmBOMB or POMB on established human neuroblastoma cell lines as a function of concentration (in  $\mu$ M).

Fig. 2 is a bar graph comparing the  $IC_{50}$  and  $IC_{90}$  values of POMB or AN-9 on the cell lines CFPAC and HT29 as assessed in the SRB assay.

5 Fig. 3 is a graphic illustration showing the *in vitro* inhibition of cellular growth by POMB and AN9 on immortalized human keratinocytes as a function of concentration (in  $\mu M$ ).

10 Fig. 4 is a graphic illustration showing the *in vitro* inhibition of cellular growth by AB, AmbOMB, POMB or BuBOMB on a primary human pancreatic adenocarcinoma cell line (BxPC-3) as a function of concentration (in  $\mu M$ ).

15 Fig. 5 is a graphic illustration showing growth inhibition measured by reduction of [ $^3H$ ]-thymidine uptake in an EBV-transformed cell line as a function of concentration of ganciclovir (GC) (panel A), GC and AB (panel B) and GC and AmbOMB (panel C). In panels B and C, the GC concentration is indicated in the inset. Concentrations are in  $\mu M$ .

20 Fig. 6 is a graphic illustration showing the percentage of human leukemic cells (HL-60) expressing the phenotypic differentiation marker CD11b for cells which had been treated with AmbOMB, POMB or AN9 for 3 days (top panel) or 6 days (bottom panel). Concentrations are in  $\mu M$ .

25 Fig. 7 is a bar graph showing the percentage of NBT-positive HL-60 cells for cells which had been treated with SB, AN9, AmbOMB or MePOMB•I. Concentrations are in  $\mu M$ .

30

#### DETAILED DESCRIPTION OF THE INVENTION

The compounds herein described may have asymmetric centers. All chiral, diastereomeric, and racemic forms are included in the present invention. Many geometric isomers of olefins and the like can also be present in

35

the compounds described herein, and all such stable isomers are contemplated in the present invention.

By "stable compound" or "stable structure" is meant herein a compound that is sufficiently robust to survive  
5 isolation to a useful degree of purity from a reaction mixture, and formulation into an efficacious therapeutic agent.

As used herein, "alkyl" means both branched- and straight-chain, saturated aliphatic hydrocarbon groups  
10 having the specified number of carbon atoms. As used herein "lower alkyl" means an alkyl group having 1 to 5 carbon atoms. As used herein, "alkenyl" means hydrocarbon chains of either a straight or branched configuration and one or more unsaturated carbon-carbon bonds, such as  
15 ethenyl, propenyl, and the like. "Lower alkenyl" is an alkenyl group having 2 to 6 carbon atoms. As used herein, "alkynyl" means hydrocarbon chains of either a straight or branched configuration and one or more carbon-carbon triple bonds, such as ethynyl, propynyl and  
20 the like. "Lower alkynyl" is an alkynyl group having 2 to 6 carbon atoms. When the number of carbon atoms is not specified, then alkyl, alkenyl and alkynyl means lower alkyl, lower alkenyl and lower alkynyl, respectively.

As used herein, "aryl" includes "aryl" and  
25 "substituted aryl." Thus "aryl" of this invention means any stable 6- to 14-membered monocyclic, bicyclic or tricyclic ring, containing at least one aromatic carbon ring, for example, phenyl, naphthyl, indanyl, tetrahydronaphthyl (tetralinyl) and the like. The  
30 presence of substitution on the aryl group is optional, but when present, the substituents can be halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro, trifluoromethyl, acylamino or carbamoyl.

As used herein, the term "heteroaryl" includes  
35 "heteroaryl" and "substituted heteroaryl." Thus "heteroaryl" of this invention means a stable 5- to 10-

5 membered monocyclic or bicyclic heterocyclic ring which is aromatic, and which consists of carbon atoms and from 1 to 3 heteroatoms selected from the group consisting of N, O and S and wherein the nitrogen may optionally be quaternized, and including any bicyclic group in which any of the above-defined heteroaryl rings is fused to a benzene ring. The heteroaryl ring may be attached to its pendant group at any heteroatom or carbon atom which results in a stable structure. The presence of

10 substitution on the heteroaryl group is optional and can be on a carbon atom, a nitrogen atom or other heteroatom if the resulting compound is stable and all the valencies of the atoms have been satisfied. When present, the substituents of the substituted heteroaryl groups are the same as for the substituted aryl groups and also include

15 alkylammonium salts when the substituent is an alkyl group attached to the nitrogen atom of the heteroaryl ring. These quaternized ammonium salts include halides, hydrohalides, sulfates, methosulfates, methanesulfates, toluenesulfates, nitrates, phosphates, maleates,

20 acetates, lactates or any other pharmaceutically acceptable salt. Examples of heteroaryl groups include, but are not limited to, pyridyl, pyrimidinyl, furanyl, thienyl, pyrrolyl, pyrazolyl, imidazolyl, tetrazolyl, benzofuranyl, benzothienyl, indolyl, indolenyl,

25 quinolinyl, isoquinolinyl and benzimidazolyl.

As used herein, "aralkyl" and "heteroaralkyl" refer to an aryl or heteroaryl group attached to an alkyl group. The aryl and heteroaryl groups of this moiety can

30 optionally be substituted in accordance with the definitions herein. Examples of heteroaralkyl groups include but are not limited to 2-, 3-, or 4-pyridylmethyl and 3-(2-, 3- or 4- pyridyl)propyl and the like.

The term "substituted", as used herein, means that

35 one or more hydrogens on the designated atom are replaced with a selection from the indicated groups, provided that



the designated atom's normal valency is not exceeded, and that the substitution results in a stable compound.

The substituents of the invention include, as indicated, halo, hydroxy, alkyl, alkoxy, amino, cyano, nitro, trifluoromethyl, aryl, heteroaryl, monoalkylamino, dialkylamino, trialkylammonium and salts thereof, carbamoyl, acylamino, arylcarbonylamino, alkoxy carbonylamino, formamido, guanidino, ureido, sulfamyl, and alkylsulfonamido. These groups can be substituents for alkyl, alkenyl, alkynyl, cycloalkyl, aryl, aralkyl, heteroaryl and heteroaralkyl groups as indicated in accordance with the invention. A "halo" group is a halogen, and includes fluoro, chloro, bromo and iodo groups. The term "alkoxy" refers to an alkyl group having at least one oxygen substituent represented by R-O-. The group "acylamino" is represented by the formula R-C(O)-NH- where R is alkyl. "Arylcarbonylamino" and "alkoxy carbonylamino" are similar to acylamino except that the R is aryl or alkoxy, respectively.

As used herein, "therapeutically-effective amount" refers to that amount necessary to administer to a host to achieve an anti-tumor effect; to induce differentiation and/or inhibition of proliferation of malignant cancer cells, benign tumor cells or other proliferative cells; to aid in the chemoprevention of cancer; to promote wound healing; to treat a gastrointestinal disorder; to treat a blood disorder or increase the hemoglobin content of blood; to modulate an immune response; to enhance recombinant gene expression; to modulate gene expression; to augment expression of tumor suppressor genes; to enhance insulin expression; to enhance chloride channel expression; to induce tolerance to an antigen; to treat, prevent or ameliorate protozoan infection; or to inhibit histone deacetylase in cells. Methods of determining therapeutically-effective amounts are well known.

When the therapeutic or effective amount of the compound is for treating, preventing or ameliorating cancer or other proliferative disorder, then that amount can be an amount effective to inhibit histone deacetylase in the subject, patient or cancerous cells. Similarly, when the therapeutic or effective amount of the compound is for treating, preventing, or ameliorating protozoan infection then that amount can be an amount effective to inhibit protozoan histone deacetylase in the subject, patient or cancerous cells.

As used herein, "pharmaceutically acceptable salts" refer to derivatives of the disclosed compounds that are modified by making acid or base salts. Examples include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids, and the like. Pharmaceutically acceptable salts include, but are not limited to, hydrohalides, sulfates, methosulfates, methanesulfates, toluenesulfonates, nitrates, phosphates, maleates, acetates, lactates and the like.

Pharmaceutically-acceptable salts of the compounds of the invention can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric or greater amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two; generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. The salts of the invention can also be prepared by ion exchange, for example. Lists of suitable salts are found in Remington's Pharmaceutical Sciences, 17th ed., Mack Publishing Company, Easton, PA, 1985, p. 1418, the disclosure of which is hereby incorporated by reference in its entirety.

The "pharmaceutical agents" for use in the methods of the invention related to the coadministration of compounds of Formula II and Formula III, include but are

not limited to anticancer agents as well as differentiating agents. For example, the pharmaceutical agent can be a cytokine, an interleukin, an anti-cancer agent, a chemotherapeutic agent, an antibody, a conjugated antibody, an immune stimulant, an antibiotic, a hormone antagonist or a growth stimulant. The pharmaceutical agent can also be a cytotoxic agent. Cytotoxic agents include antiviral nucleoside antibiotics such as ganciclovir, acyclovir, and famciclovir. Cytotoxic agents can also include radiation therapy.

As used herein, the "chemotherapeutic agents" include but are not limited to alkylating agents, purine and pyrimidine analogs, vinca and vinca-like alkaloids, etoposide and etoposide-like drugs, corticosteroids, nitrosoureas, antimetabolites, platinum-based cytotoxic drugs, hormonal antagonists, anti-androgens and antiestrogens.

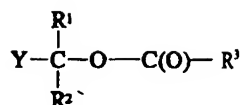
The "cytokines" for use herein include but are not limited to interferon, preferably  $\alpha$ ,  $\beta$  or  $\gamma$  interferon, as well as IL-2, IL-3, G-CSF, GM-CSF and EPO.

As used herein, an "immune stimulant" is a substance such as *C. parvum* or sarcolectin which stimulates a humoral or cellular component of the immune system.

The chemotherapeutic agents of the invention include but are not limited to tamoxifen, doxorubicin, L-asparaginase, dacarbazine, amsacrine, procarbazine, hexamethylmelamine, mitoxantrone and gemcitabine.

#### SYNTHETIC METHODS

The compounds of the present invention can generally be prepared by any method known in the art. For example, the compounds of the invention can be made by reacting the acid RCOOH with a reagent of the formula



or by similar reactions between any of the appropriate acids and the appropriate alkyl halides in the presence of a base, where Y is a leaving group such as halogen, methanesulfonate or *p*-toluenesulfonate and R, R<sup>1</sup>, R<sup>2</sup> and R<sup>3</sup> are as defined herein. The above reagents are readily prepared according to literature procedures, see for example, Nudelman et al., J. Med. Chem. 35:687-694, 1992, and Japanese patent 07033709 (1995). The base can be a trialkylamine, pyridine, an alkali metal carbonate or other suitable base. The reaction can be carried out in the presence or absence of an inert solvent. Suitable solvents include, for example, acetone, benzene, toluene, tetrahydrofuran, ethyl acetate, acetonitrile, dimethylformamide, dimethyl sulfoxide, chloroform, dioxan or 1,2-dichloroethane.

The procedures outlined above can be improved by one skilled in the art by, for instance, changing the temperature, duration, stoichiometry or other parameters of the reactions. Any such changes are intended to fall within the scope of this invention.

#### ACTIVITY

The activities of the compounds of the invention can be measured using generally-accepted techniques known to those skilled in the art consistent with the activity of interest. For example, the activity of compounds useful as differentiating agents can be measured using standard methodology of the nitro-blue tetrazolium reduction assay (e.g., Rabizadeh et al., FEBS Lett. 328:225-229, 1993; Chomienne et al., Leuk. Res. 10:631, 1986; and Breitman et al. in Methods for Serum-free Culture of Neuronal and

Lymphoid Cells, Alan R. Liss, NY, p. 215-236, 1984 which are hereby incorporated by reference in their entirety) and as described below. This *in vitro* assay has been deemed to be predictive and in fact correlative with *in vivo* efficacy (Castaigne et al., Blood 76:1704-1709, 1990).

Another assay which is predictive of differentiating activity is the morphological examination for the presence of Auer rods and/or specific differentiation cell surface antigens in cells collected from treatment groups, as described in Chomienne et al., (Blood 76:1710-1717, 1990 which is hereby incorporated by reference in its entirety) and as described below.

The compounds of the present invention also have anti-proliferative and anti-tumor activity. The anti-proliferation activity of compounds of the present invention can be determined by methods generally known to those skilled in the art. Generally-accepted assays for measuring viability and anti-proliferative activity are the trypan blue exclusion test and incorporation of tritiated thymidine, also as described by Chomienne, et al., above, which is incorporated herein by reference. Other assays which predict and correlate antitumor activity and *in vivo* efficacy are the human tumor colony forming assay described in Shoemaker et al., Can. Res. 45:2145-2153, 1985, and inhibition of telomerase activity as described by Hiyayama et al., J. Natl. Cancer Inst. 87:895-908, 1995, which are both incorporated herein by reference in their entirety. These assays are described in further detail below.

#### Cell Cultures

Human promyelocytic leukemia cells (HL-60), human pancreatic carcinoma cells (PaCa-2) and human breast adenocarcinoma cells, pleural effusion cells (MCF-7) can be cultured as follows. Cells are grown in RPMI media

with 10% FCS, supplemented with 2 mM glutamine and incubated at 37°C in a humidified 5% CO<sub>2</sub> incubator. Alternatively, cells can be grown in any other appropriate growth medium and conditions which supports the growth of the cell line under investigation. Viability can be determined by trypan blue exclusion. Cells are exposed to a test compound, cultures are harvested at various time points following treatment and stained with trypan blue.

#### Cellular Staining to Detect Differentiation

Lipid staining and/or immunochemical staining of casein can be used as a marker for cellular differentiation of breast cancer cells (Bacus et al., Md. Carcin. 3:350-362, 1990). Casein detection can be done by histochemical staining of breast cancer cells using a human antibody to human casein as described by Cheung et al., J. Clin. Invest. 75:1722-1728, which is incorporated by reference in its entirety.

#### Nitro-Blue Tetrazolium (NBT) Assay:

Cell differentiation of myeloid leukemia cells can be evaluated, for example, by NBT reduction activity as follows. Cell cultures are grown in the presence of a test compound for the desired time period. The culture medium is then brought to 0.1% NBT and the cells are stimulated with 400 mM of 12-O-tetradecanoyl-phorbol-13-acetate (TPA). After incubation for 30 min at 37°C, the cells are examined microscopically by scoring at least 200 cells. The capacity for cells to reduce NBT is assessed as the percentage of cells containing intracellular reduced black formazan deposits and corrected for viability.

#### Cell Surface Antigen Immunophenotyping

Cell surface antigen immunotyping can be conducted using dual-color fluorescence of cells gated according to size. The expression of a panel of antigens from early myeloid (CD33) to late myeloid can be determined as described in Warrell, Jr. et al., New Engl. J. Med. 324:1385-1392, 1992, which is incorporated by reference herein in its entirety.

#### Apoptosis Evaluation

Apoptosis can be evaluated by DNA fragmentation, visible changes in nuclear structure or immunocytochemical analysis of Bcl-2 expression.

DNA fragmentation can be monitored by the appearance of a DNA ladder on an agarose gel. For example, cellular DNA is isolated and analyzed by the method of Martin et al., J. Immunol., 145:1859-1867, 1990 which is incorporated by reference herein in its entirety.

Changes in nuclear structure can be assessed, for example, by acridine orange staining method of Hare et al., J. Hist. Cyt., 34:215-220, 1986 which is incorporated by reference herein in its entirety.

Immunological detection of Bcl-2 can be performed on untreated cells and cells treated with the test compound. HL-60 cells are preferred but other cell lines capable of expressing Bcl-2 can be used. Cytospins are prepared and the cells are fixed with ethanol. Fixed cells are reacted overnight at 4°C with the primary monoclonal antibody, anti-Bcl-2 at a dilution of 1:50. Staining is completed to visualize antibody binding, for example, using Strep A-B Universal Kit (Sigma) in accordance with the manufacturer's instructions. Identically-treated cells which received no primary antibody can serve as a non-specific binding control. Commercial kits are also available and can be used for detecting apoptosis, for example, Oncor's Apop Tag®.

Modulation of Gene Expression

The levels of expression from oncogene and tumor suppressor genes can be evaluated by routine methods known in the art such as Northern blotting of RNA, immunoblotting of protein and PCR amplification.

Mouse Cancer Model

Compounds can be examined for their ability to increase the life span of animals bearing B16 melanomas, Lewis lung carcinomas and myelomonocytic leukemias as described in Nudelman et al., J. Med. Chem. 35:687-694, 1992, or Rephaeli et al., Int. J. Cancer 49:66-72, 1991, which are incorporated by reference herein in their entireties.

For example, the efficacy of compounds of the present invention in a leukemia model can be tested as follows: Balb/c mice are injected with WEHI cells and a test compound or control solution is administered the following day. The life span of the treated animals is compared to that of untreated animals.

The efficacy of compounds of the present invention on primary tumors can also be tested with subcutaneously implanted lung carcinoma or B16 melanoma by measuring the mass of the tumor at the site of implantation every two weeks in control and treated animals.

The efficacy of compounds in xenografts can be determined by implanting the human tumor cells subcutaneously into athymic mice. Human tumor cell lines which can be used include, but are not limited to, prostate carcinoma (human Pc-3 cells), pancreatic carcinoma (human Mia PaCa cells), colon adenocarcinoma (human HCT-15 cells) and mammary adenocarcinoma (human MX-1 cells). Treatment with control solution or a test compound of the invention begins, for example, when tumors are approximately 100 mg. Anti-tumor activity is assessed by measuring the delay in tumor growth, and/or



tumor shrinking and/or increased survival of the treated animals relative to control animals.

#### Telomerase Activity

5           High levels of telomerase activity is associated with the high proliferation rate found in cancer cells. Compounds which inhibit telomerase activity results in inhibition of cancer cell growth and de-differentiation. Commercially available telomerase assays can thus be used  
10           to assess the anticancer activities of compounds on cancer cell lines.

#### Chemoprevention

15           The chemoprevention activity of the compounds of the invention can be determined in the two-stage mouse carcinogenesis model of Nishimo et al. (supra). In this regard, AmbOMB is expected to have good chemoprevention activity since it can release two active compounds when hydrolyzed by cellular or blood esterases, namely butyric  
20           acid and para-aminobenzoic acid (PABA). PABA is associated with antimutagenic activity. (Semenov et al., 1994, Vopr. Med. Khim. 40:48).

#### Assay of Compounds

25           Compounds of the invention, their salts or metabolites, can be measured in a biological sample by any method known to those skilled in the art of pharmacology, clinical chemistry or the like. Such methods for measuring these compounds are standard  
30           methods and include, but are not limited to high performance liquid chromatography (HPLC), gas chromatography (GC), gas chromatography mass spectroscopy (GC-MS), radioimmunoassay (RIA), and others.

Dosage and Formulation

The compounds of the present invention can be administered to a mammalian patient to treat cancer or in any other method of the invention which involves treating a patient by any means that produces contact of the active agent with the agent's site of action in the body of the subject. Mammalian patients include humans and domestic animals. The compounds of the invention can be administered by any conventional means available for use in conjunction with pharmaceuticals, either as individual therapeutic agents or in a combination of therapeutic agents. The compounds can be administered alone, but are generally administered with a pharmaceutical carrier selected on the basis of the chosen route of administration and standard pharmaceutical practice. The pharmaceutical compositions of the invention may be adapted for oral, parenteral, transdermal, transmucosal, rectal or intranasal administration, and may be in unit dosage form, as is well known to those skilled in the pharmaceutical art. The term "parenteral" as used herein includes subcutaneous, intravenous, intramuscular, or intrasternal injection or infusion techniques.

The appropriate dosage administered in any given case will, of course, vary depending upon known factors, such as the pharmacodynamic characteristics of the particular agent and its mode and route of administration; the age, general health, metabolism, weight of the recipient and other factors which influence response to the compound; the nature and extent of the symptoms; the kind of concurrent treatment; the frequency of treatment; and the effect desired. A daily dosage of active ingredient can be expected to be about 10 to 10,000 milligrams per meter<sup>2</sup> of body mass (mg/m<sup>2</sup>), with the preferred dose being 50-5,000 mg/m<sup>2</sup> body mass.

Dosage forms (compositions suitable for administration) contain from about 1 mg to about 1 g of

active ingredient per unit. In these pharmaceutical compositions the active ingredient will ordinarily be present in an amount of about 0.5-95% by weight based on the total weight of the composition.

5           The active ingredient can be administered orally in solid or semi-solid dosage forms, such as for example hard or soft-gelatin capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, disperse  
10           powders or granules, emulsions, and aqueous or oily suspensions. It can also be administered parenterally, in sterile liquid dosage forms. Other dosage forms include transdermal administration via a patch mechanism or ointment.

          Compositions intended for oral use may be prepared  
15           according to any methods known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents including sweetening agents, flavoring agents, coloring agents, and preserving agents in order to provide a pharmaceutically  
20           elegant and palatable preparation.

          Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. Such excipients may include, for example, inert diluents, such  
25           as calcium phosphate, calcium carbonate, sodium carbonate, sodium phosphate, or lactose; granulating disintegrating agents, for example, maize starch or alginic acid; binding agents, such as starch, gelatin, or acacia; and lubricating agents, for example, magnesium  
30           stearate, stearic acids or talc. Compressed tablets may be uncoated or may be sugar coated or film coated by known techniques to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric coated for selective disintegration and adsorption in the  
35           gastrointestinal tract.

Hard gelatin capsules or liquid filled soft gelatin capsules contain the active ingredient and inert powdered or liquid carriers, such as, but not limited to calcium carbonate, calcium phosphate, kaolin, lactose, lecithin starch, cellulose derivatives, magnesium stearate, stearic acid, arachis oil, liquid paraffin, olive oil, pharmaceutically-accepted synthetic oils and other diluents suitable for the manufacture of capsules. Both tablets and capsules can be manufactured as sustained release-products to provide for continuous release of medication over a period of hours.

Aqueous suspensions contain the active compound in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, e.g., sodium carboxymethylcellulose, methylcellulose, hydroxypropylmethylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth, and gum acacia; dispersing or wetting agents, such as a naturally occurring phosphatide, e.g., lecithin, or condensation products of an alkylene oxide with fatty acids, for example of polyoxyethylene stearate, or a condensation products of ethylene oxide with long chain aliphatic alcohols, e.g., heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol, e.g., polyoxyethylene sorbitol monooleate, or a condensation product of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, e.g., polyoxyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl or *n*-propyl *p*-hydroxy benzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose, saccharin, or sodium or calcium cyclamate.

Dispersable powders and granules suitable for preparation of an aqueous suspension by the addition of

water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example, sweetening, flavoring, and coloring agents, can also be present.

Syrups and elixirs can be formulated with sweetening agents, such as glycerol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents.

The pharmaceutical compositions can be in the form of a sterile injectable preparation, for example, as a sterile injectable aqueous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation can also be a sterile injectable solution or suspension in a nontoxic parenterally-acceptable diluent or solvent, for example, as a solution in 1,3-butanediol.

In general, water, a suitable oil, saline, aqueous dextrose (glucose), polysorbate and related sugar solutions, emulsions, such as Intralipid® (Cutter Laboratories, Inc., Berkley, CA) and glycols such as propylene glycol or polyethylene glycols are suitable carriers for parenteral solutions. Antioxidizing agents, such as but not limited to sodium bisulfite, sodium sulfite, or ascorbic acid, either alone or combined, are suitable stabilizing agents. Also used can be citric acid and its salts and sodium EDTA. In addition, parenteral solutions can contain preservatives, such as but not limited to benzalkonium chloride, methyl- or propyl-paraben, and chlorobutanol.

The pharmaceutical compositions of the present invention also include compositions for delivery across

cutaneous or mucosal epithelia including transdermal, intranasal, sublingual, buccal, and rectal administration. Such compositions may be part of a transdermal device, patch, topical formulation, gel, etc., with appropriate excipients. Thus, the compounds of the present invention can be compounded with a penetration-enhancing agent such as 1-n-dodecylazacyclopentan-2-one or the other penetration-enhancing agents disclosed in U.S. Patent Nos. 3,991,203 and 4,122,170 which are hereby incorporated by reference in their entirety to describe penetration-enhancing agents which can be included in the transdermal or intranasal compositions of this invention.

Suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences, Mack Publishing Company, a standard reference text in this field, which is incorporated herein by reference in its entirety.

Various modifications of the invention in addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The foregoing disclosure includes all the information deemed essential to enable those skilled in the art to practice the claimed invention. Because the cited patents or publications may provide further useful information these cited materials are hereby incorporated by reference in their entirety.

## EXAMPLE 1

Synthesis of BuBuOMB

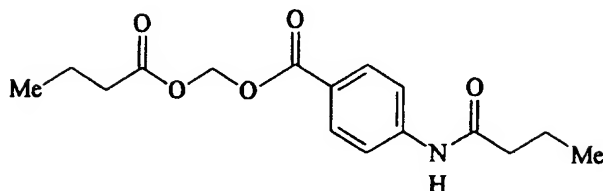
The synthesis of BuBuOMB was done as follows: To a  
5 solution of 4-butyramidobutyric acid (1.56 g, 9 mmol) in  
dry dimethylformamide (DMF) (6 mL), at room temperature  
under nitrogen, were added chloromethyl butyrate (1.26 g,  
1 eq) and triethylamine (1.5 mL, 1.1g, 1.2 eq). The  
reaction mixture was heated to 65°C for 3 h whereby a  
10 large amount of precipitate formed and TLC (CHCl<sub>3</sub>:MeOH  
7:1, detection-vanillin) showed that most of the acid had  
reacted. The precipitate was filtered and washed with  
ethyl acetate. The filtrate was partitioned between  
water and ethyl acetate. The aqueous phase was extracted  
15 back with a small amount of ethyl acetate, and the  
combined organic phase was washed three times with water,  
three times with a 5% solution of NaHCO<sub>3</sub>, and twice with  
brine, dried with MgSO<sub>4</sub>, and evaporated to give the crude  
product as a yellowish semisolid (1.6 g, 65% yield) which  
20 was chromatographed on silica gel (60 g, ethyl  
acetate:hexane:isopropanol 8:8:1). The product was found  
in the second fraction. The pure product (1 g, 40%  
yield) was a colorless oil.

## EXAMPLE 2

Synthesis of BuBOMB

BuBOMB has the following structure:

5



and was synthesized as follows. To a stirred solution of  
4-butyramidobenzoic acid (3.33 g, 16.1 mmol) and  
chloromethyl butyrate (2.2 g, 1 eq) in dry DMF (10 mL),  
under nitrogen was dropwise added triethylamine (2.7 mL,  
1.95 g, 1.2 eq). The reaction mixture was heated at 60°C  
for 3 h, during that time a large amount of precipitate  
formed and TLC (ethyl acetate:hexane 1:1) showed that  
most of the acid has reacted. The precipitate was  
filtered and washed with ethyl acetate. The filtrate was  
partitioned between water and ethyl acetate. The aqueous  
phase was washed with a small amount of ethyl acetate,  
and the combined organic phase was washed with water  
(3x), 5% solution of NaHCO<sub>3</sub> (2x), brine (2x), dried with  
MgSO<sub>4</sub> and evaporated to give the crude product as a solid  
(3.3 g), which was crystallized from ethyl acetate-  
hexane to give a white solid (2.54 g, 77% yield), mp 89-  
90°C.

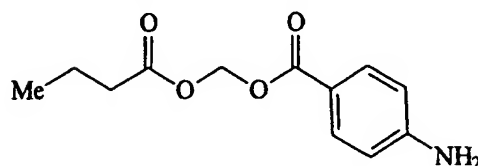


## EXAMPLE 3

Synthesis of AmBOMB

AmBOMB has the following structure:

5



and was synthesized as follows. To a stirred solution of chloromethyl butyrate (21.5 g, 157 mmol) in DMF (60 mL), under nitrogen, 4-aminobenzoic acid (21.05 g, 153 mmol), was added, followed by the dropwise addition of triethylamine (25.5 mL, 18.5 g, 1.2 eq) until the acid dissolved. The reaction mixture was heated at 60°C overnight, during that time a large amount of precipitate formed and TLC (ethyl acetate:hexane 2:1) showed that most of the acid has reacted. The precipitate was filtered and washed with ethyl acetate. The solution was partitioned between water and ethyl acetate. The aqueous phase was extracted with a small amount of ethyl acetate, and the combined organic phase was washed with a 5% solution of NaHCO<sub>3</sub> (3x). The organic phase was washed with water (2x), brine (2x) and dried with MgSO<sub>4</sub>. Evaporation of the solvent gave a pasty residue which was triturated with hexane to give the product as white solid (18 g) and was crystallized from isopropanol. The first crop gave 6.7 g of white product. A second crop, obtained by concentrating the mother liquor gave an additional 3 g of material of the same quality. Additional crops of non-crystalline powdery material (5 g) were obtained by triturating, in a small amount of isopropanol, the residues upon evaporation of the mother liquors. The

40

total purified product obtained was 15 g (41% yield), mp  
130-132°C.

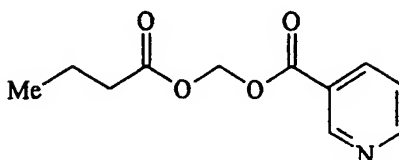
41

## EXAMPLE 4

Synthesis of POMB

POMB has the following structure:

5



and was synthesized as follows. To a stirred solution of chloromethyl butyrate (26 g, 190 mmol) dissolved in DMF (90 mL), under nitrogen, nicotinic acid (23.4 g, 190 mmol), was added, followed by the dropwise addition of triethylamine (32 mL, 23.23 g, 1.2 eq) until the acid dissolved. The reaction mixture was heated at 60°C overnight, during that time a large amount of precipitate formed and TLC (ethyl acetate:hexane 2:1) showed that most of the acid was consumed. The precipitate was filtered and washed with ethyl acetate (300 mL). The solution was washed with water (4x), the aqueous phase was extracted with a small amount of ethyl acetate (3x10 mL) and the combined organic phase was washed with brine (2x), dried over MgSO<sub>4</sub> and evaporated. The product was obtained as a brownish oil (31.7 g), which was distilled at 109-110°C (0.05 mm Hg) to give a colorless liquid (25 g, 59% yield).

## EXAMPLE 5

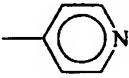
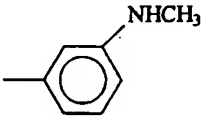
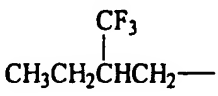
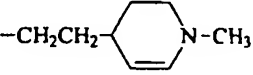
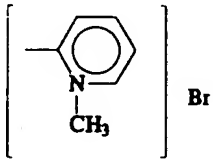
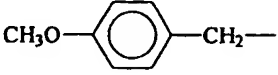
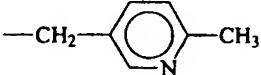
Synthesis of MePOMB•I and Additional Compounds

5 MePOMB•I, the iodide salt of MePOMB, was synthesized  
by adding methyl iodide (MeI) (1.55 mL, 3.55 g, 25 mmol)  
to a solution of 3-pyridoyloxymethyl butyrate (975 mg,  
4.4 mmol) in dry acetone (100 mL). The reaction mixture  
was refluxed for 20 h and a fine precipitate was formed  
which was filtered and washed with acetone. The filtrate  
10 was evaporated to give the product, MePOMB•I. The  
chloride salt can be made, for example, by stirring  
MEPOMB•I with silver chloride in an inert solvent such as  
acetone, followed by the removal of the silver iodide.

15 Additional compounds of the invention are provided  
in Table I. These compounds are those of Formula I or Ia  
having the designated groups. These compounds can be  
synthesized in a manner analogous to Examples 1-5 or as  
provided in the Detailed Description of the Invention.

43

TABLE 1  
Additional Compounds

R	R <sup>1</sup>	R <sup>2</sup>	R <sup>3</sup>
CH <sub>2</sub> =CHCH <sub>2</sub> —	H	H	
ClCH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> —	CH <sub>3</sub>	H	
	CH <sub>3</sub>	CH <sub>3</sub>	
C <sub>6</sub> H <sub>5</sub> (CH <sub>2</sub> ) <sub>3</sub> —	C <sub>3</sub> H <sub>7</sub>	H	
2-pyridyl-CH <sub>2</sub> -	H	H	—CH <sub>2</sub> CH <sub>2</sub> CH <sub>2</sub> N(CH <sub>3</sub> ) <sub>2</sub>
	C <sub>3</sub> H <sub>7</sub>	H	

## EXAMPLE 6

Clonogenicity of Established Tumor Cell Lines

5 Inhibition of tumor growth was tested using cell lines as follows:

The cell lines listed in Table 2 were grown to 70-80% confluence in complete media [RPMI 1640 containing 10% FCS, 100 IU penicillin, 100 µg/mL streptomycin and 2 mM L-glutamine]. Cells were harvested, washed in  
10 complete media, and counted. Cell viability was determined by trypan blue exclusion. The cells were placed into soft agar (0.12% in media) and plated at 5,000 viable cells per well onto an agarose underlayer (0.4%) in 24-well plates. After overnight culture, AB, POMB or AmbOMB was added at the indicated concentration.  
15 Control cells received media alone. As a control for cell death, cells were treated with a superlethal dose of 10 µg/ml of cisplatin. The dosage of AB, POMB or AmbOMB which inhibited fifty percent (or ninety percent) of cell proliferation ( $IC_{50}$  or  $IC_{90}$ ) was calculated using the Chou  
20 Analysis' Median Effective Dose equation.

Clonogenicity is determined as the percentage of clones in treated cultures relative to clones in media-treated control cultures. A representative clonogenicity  
25 titration curve for AB, POMB and AmbOMB is shown with four neuroblastoma cell lines in Fig. 1. The  $IC_{50}$  and  $IC_{90}$  values of AB, POMB and AmbOMB for cancer cell lines are provided in Table 3.

The results demonstrate that POMB and AmbOMB are  
30 more potent growth inhibitors than AB. The data show that POMB, AmbOMB and AB inhibit cell proliferation in a dose-dependent manner but that the cells are at least an order of magnitude more sensitive to POMB and AmbOMB. The ratio of  $IC_{50}$  AB: $IC_{50}$  AmbOMB ranges between  
35 6- to 56-fold with a median of 21 µM and the ratio of  $IC_{50}$  AB: $IC_{50}$  POMB ranges between 3.7- to 81-fold with a median

of 21  $\mu\text{M}$ . Similarly the ratio of  $\text{IC}_{90}$  AB: $\text{IC}_{90}$  AmBOMB ranges between 14- to 791-fold with a median value of 45  $\mu\text{M}$  and the ratio of  $\text{IC}_{90}$  AB: $\text{IC}_{90}$  POMB ranges between 16- to 277-fold with a median value of 47  $\mu\text{M}$ . The  $\text{IC}_{90}$  values  
5 are clinically important for assessing eradication of residual cancer disease.

TABLE 2  
Human Tumor Cell Lines

	Cell Lines	Origin
5	MCF7-WT	Breast Carcinoma
	MCF7-40F	Breast Carcinoma
	PC3	Prostate Carcinoma
	LNCaP	Prostate Carcinoma
	K-562	Erythroleukemia
10	SK-N-SH	Neuroblastoma
	NBAS-5	Neuroblastoma
	IMR-32	Neuroblastoma
	LA1-5S	Neuroblastoma
	NBL-W-N	Neuroblastoma
15	SMS-KAN	Neuroblastoma
	SK-N-MC	Neuroblastoma
	NGP	Neuroblastoma
	SMS-KCN	Neuroblastoma



**TABLE 3**  
**Inhibition of Established and Primary Tumor**  
**Cell Lines by AB, AmBOMB and POMB**

Cell Line	AB		AmBOMB		Ratio AB/AmBOMB		POMB		Ratio AB/POMB	
	IC <sub>50</sub> <sup>(a)</sup>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>	IC <sub>50</sub>	IC <sub>90</sub>
MCF7-WT	1500	9473	42	112	35.7	84.6	44	84	34.1	112.8
MCF7-40F	817	3183	40	83	20.4	38.3	51	195	16	16.3
PC3	226	4478	23	188	9.8	23.8	61	186	3.7	24.1
LNCaP	780	6703	35	90	22.3	74.5	53	143	14.7	46.9
K562	772	6658	43	156	18	42.7	57	141	13.5	47.2
SK-N-SH	998	3397	40	62	25	54.8	31	99	32.2	34.3
NBAS-5	883	13030	21	44	42	296.1	20	47	44.2	277.2
SK-N-MC	215	1314	26	91	8.3	14.4	11	25	19.5	52.6
IMR-32	881	3566	21	46	42	77.5	27	69	32.6	51.7
NGP	197	1622	33	83	6	19.5	15	40	13.1	40.6
LAL-5S	1627	2675	29	59	56.1	45.3	20	33	81.4	81.1
SMS-KCN	1872	NA	89	NA	21	NA	99	739	18.9	NA
NBL-W-N	489	3074	47	154	10.4	20	22	47	22.2	65.4
SMS-KAN	1138	2079	49	152	23.2	791.6	26	57	43.8	36.5
Primary <sup>(b)</sup>	464	2120	32	57	13.5	37	41	97	11	22

(a) All concentrations are in  $\mu\text{M}$ .

(b) Data from 53 primary tumor cell lines treated as described in Example 3.

## EXAMPLE 7

Inhibition of Clonogenicity of Primary Tumor Cells

5       The effect of AmBOMB and POMB was compared to that  
of AB on a variety of primary tumor cells as described in  
Example 2, except that cells were seeded at 30,000 cells  
per well. Table 3 provides the average  $IC_{50}$  value and  $IC_{90}$   
value for each compound as well as the  $IC_{50}$  and  $IC_{90}$  ratio  
of each compound to AB. The tested tumor cells were from  
10   11 non-small cell lung carcinomas, 10 breast carcinomas,  
10 gastric carcinomas, 10 ovarian carcinomas, and 10 CNS  
tumors. The results demonstrate that AmBOMB and POMB are  
more potent inhibitors of cell proliferation than is AB,  
especially for lung, breast, ovarian, CNS and  
15   gastrointestinal cancers.

## EXAMPLE 8

Inhibition of Cancer Cell ProliferationAssessed by the SRB Assay

5           The inhibition of cell proliferation was measured in  
the indicated cancer cell lines using the sulforhodamine  
B (SRB) assay as described by Monks et al., 1991, J. Natl.  
Can. Inst. 83:757-766. The SRB assay is used to screen  
for anti-cancer drugs. A comparison of POMB, AB and AN-9  
10 demonstrates that POMB exerts at least 100-fold greater  
activity than AB as measured by the IC<sub>50</sub> and IC<sub>90</sub> values  
(Table 4). POMB was also 1.2- to 1.5-fold more active  
than AN-9 as determined by the IC<sub>50</sub> and IC<sub>90</sub> values.  
(Table 4 and Fig. 2).

Table 4

Comparison of POMB, AB and AN-9 in the SRB Assay

AGENT	CFPAC		HT29	
	IC <sub>50</sub> *	IC <sub>90</sub> *	IC <sub>50</sub>	IC <sub>90</sub>
AB	>4.000	>4.000	>4.000	>4.000
POMB	0.037	0.0604	0.103	0.198
25 AB/POMB	108	66.22	39	20.250
AN9	0.0559	0.0898	0.144	0.231
AN9/POMB	1.51	1.48	1.4	1.160

## EXAMPLE 9

Effect POMB and AN-9 on Hyperproliferating Cells

5       The SRB assay was used to compare the effect of POMB  
and AN-9 on the proliferation of hyperproliferating,  
immortalized human keratinocytes as described in Example  
8 (Fig. 3). The  $IC_{50}$  and  $IC_{90}$  of POMB on the keratinocytes  
was 0.032 and 0.058, respectively, whereas for AN-9 the  
same parameters were 0.108 and 0.221, respectively.

10

## EXAMPLE 10

Clonogenicity of Human Pancreatic Cells

5       The effect of 24 h exposure to POMB, AmBOMB, BuBOMB,  
and AB was determined by a colony-forming assay on the  
pancreatic cell line BxPC-3, a primary adenocarcinoma  
ACTTnr:CRL-1687. Freshly trypsinized cells were plated  
at 500 cells/dish in 60 mm<sup>2</sup> tissue culture dishes with the  
indicated concentration of each compound and incubated  
10   for 24 h at 37°C in 5% CO<sub>2</sub>/air atmosphere. The cultures  
were washed with PBS, fresh medium was added, and the  
cultures were incubated for 7-12 days to allow the  
formation of colonies. Colonies were fixed with  
methanol, stained with Giemsa and counted. All  
15   incubations were performed in triplicate.

      The results shown in Fig. 4 demonstrate that 24 h  
treatment with POMB, AmBOMB and BuBOMB caused a dose-  
dependent growth inhibition, with complete inhibition  
occurring at concentrations above 50  $\mu$ M. In contrast, a  
20   100  $\mu$ M dose of AB did not cause complete growth  
inhibition of the cells.

## EXAMPLE 11

Synergy of AB and AmBOMB with Antiviral Agent

5       The effect of AmBOMB to AB was compared on EBV  
virus-associated tumors. EBV-positive cells from P3HR-1,  
a human lymphoma cell line, were incubated in growth  
medium with the specified concentration of AmBOMB, AB,  
ganciclovir (GC), or combination of these, for 72 hours,  
seeded in 96-well plates and pulsed with 1  $\mu$ Ci [ $^3$ H]-  
10   thymidine/well during the last 16 hours of exposure to  
the compounds. Cells were harvested with a cell  
harvester, using glass microfiber filters. The  
incorporation of [ $^3$ H]-thymidine was determined by  
retention of the acid insoluble fraction on filters and  
15   is expressed as CPM. The results of a representative  
experiment are shown in Fig. 5. Fig. 5A shows a  
titration curve using GC; Fig. 5B shows titration curves  
using GC and AB; and Fig. 5C shows similar titration  
curves for GC and AmBOMB; the GC concentration is  
20   indicated in the inset of panels B and C.

      The results show that AmBOMB is about as potent as  
AB in inhibiting EBV-positive lymphoma cells, but  
importantly, that AB and AmBOMB interact synergistically  
with GC to inhibit proliferation of an EBV-associated  
25   tumor cell line.

## EXAMPLE 12

Inducing Differentiation

Cancer cell differentiation was evaluated in a human  
5 leukemia cell line by nitroblue tetrazolium reduction  
(NBT) activity (Koeffler, Blood, 62: 709-721, 1983) or by  
changes in expression of myelocytic maturation marker  
CD11b. Differentiation was also evaluated in a breast  
10 carcinoma cell line by lipid staining (Bacus et al., Mol.  
Carcinog. 3:350-362, 1990).

The level of CD11b was measured on HL-60 cells by  
flow cytometry using a monoclonal antibody (MAb) against  
CD11b in a standard indirect immunofluorescence assay.  
Cells were cultured for 3 or 6 days with the indicated  
15 concentration of AmbOMB, POMB or AN-9. Cultured cells  
were collected by centrifugation, resuspended at  $10^6$  cells  
per  $20\mu$  RPMI+10% FCS and incubated with MAb for 30 min at  
40°C. The cells were washed twice in cold PBS + 10% FCS  
and incubated with a 1:20 dilution of FITC-conjugated  
20 F(ab')<sup>2</sup> fragment of rabbit anti-mouse IgG for 20-30 min at  
40°C in the dark. After washing the cells twice in cold  
PBS + 10% FCS, the flow cytometry was performed. The  
results are shown in Fig. 6. AmbOMB was the most active  
differentiation inducer and AN-9 was the least active.

The differentiation ability of AmbOMB and MePOMB•I  
25 with the human leukemia cell line HL-60 was compared to  
AN-9 and SB. Briefly, HL-60 cells were incubated with  
the indicated concentration of compound for three days,  
washed, resuspended in saline containing 0.1% NBT and  
30 stimulated with 0.4  $\mu$ M phorbol ester for 30 minutes at  
37°C. The cells were examined microscopically and at  
least 200 cells were scored. The results are provided  
graphically in Fig.7 and demonstrate that AmbOMB and  
MePOMB•I are substantially more active at inducing  
35 differentiation than SB. In general AmbOMB and MePOMB•I

are much more active than AN-9 at comparable concentrations.

5 In the case of the human breast carcinoma cell line Au565, 100% of the cells treated with 10  $\mu$ M POMB were positive for lipid staining whereas less than 1% of cells treated with the same concentration of AB were positive for lipid staining. In MCF-7 cells, another breast carcinoma cell line, 50% of the cells were positive for lipid staining at 50  $\mu$ M, a concentration at which AB did  
10 not induce differentiation.



## EXAMPLE 13

Induction of Apoptosis

Apoptosis was measured as described by Telford et al., 1991, Cell Physiol. 24:447-459, in HL-60 cells and in the human erthroblastic cell line, K-562. Cells were treated with AmbOMB•HCl or POMB for 3 days, fixed, stained with propidium iodine and analyzed by flow cytometry for cell cycle distribution. A distinct subpopulation of cells was observed in a region below the G0-G1 cells. This region consisted of cells with fragmented DNA. The increase in apoptotic cells in HL-60 and K-562 cells lines treated with varying concentrations of AmbOMB•HCl or POMB is shown in Table 4.

Induction of apoptosis by POMB and AB in MCF-7 cells was evaluated by staining cells with the WAF antibody and by Western blot analysis as described by Bacus et al., 1996, Oncogene 12:2535-2547. The percentage of cells expressing WAF was 14% in the controls. The results demonstrate that POMB induces apoptosis in a dose-dependent manner and that POMB induces apoptosis at a 10-fold lower concentration and to a greater extent than does AB (Table 5).

TABLE 4

Induction of Apoptosis in Human Leukemia Cell Lines

Compound	Concentration	% Apoptotic Cells	% Apoptotic Cells
	( $\mu$ M)	HL-60	K562
AmBOMB•HCl	55	10.5	11.2
	110	20.6	15
	165	76	20.3
POMB	12.5	12.4	9.7
	25	80	73
	37.5	79	82

Table 5

Apoptosis in Human Breast Cancer Cells

Concentration ( $\mu$ M)	AB*	POMB*
10	ND**	21
25	ND	19
50	ND	59
100	23	56

\* Percentage of cells expressing the WAF protein.

\*\* ND, not detected.

## EXAMPLE 14

Induction of Hemoglobin Synthesis

Hb Measurement: Hemoglobin (Hb) was measured by  
5 benzidine staining of K562 cells after 5 days exposure to  
AmBOMB, POMB, BuBOMB or AB according to the procedure of  
Fibach *et al.* (1993), *infra*. Quantitative measurement of  
HbF in K562 culture or human erythroid cultures was  
determined by ion-exchange high pressure liquid  
10 chromatography (HPLC) as described by Fibach *et al.*,  
Blood 81:1630-1635, 1993.

K562 cells: K562 is an erthroblast cell line that  
develops some properties of erythroid, megakaryocyte or  
monocyte cells, depending on the specific stimulus, when  
15 induced by different chemicals. K562 cells were grown in  
RPMI with 10% FCS, supplemented with 2 mM glutamine.  
cells were incubated at 37°C in a humidified, 5% CO<sub>2</sub>  
incubator.

Treatment of K562 cells with AmBOMB, POMB, BuBOMB or  
20 AB showed that, on a molar basis, the three compounds of  
the invention had higher activity in inducing erythroid  
differentiation (hemoglobin accumulation) than did AB.  
This was evident from the higher proportion of Hb-  
containing cells per the total cell population (Table 6)  
25 as well as the total Hb content of the cultures (Table  
7). The extent of differentiation of the treated  
cultures was directly related to the drug dose. The  
diluent, DMF and water, had no effect on cell growth,  
cell viability or differentiation.

30 Erythroid cells: Erythroid cells were isolated from  
peripheral blood of healthy individuals by the method of  
Fibach (1993). The results showed that BuBOMB, POMB and  
BuBuOMB increased HbF in cultured erythroid cells  
relative to untreated control cultures (Table 8).

TABLE 6

Percentage K562 Cells Containing HbF

Concentration ( $\mu$ M)	AB	AmBOMB	POMB	BuBOMB
10	ND**	17	5.3	4.7
50	3 $\pm$ 2	-	13	11
100	ND	-	24.7	22.3

\*\* ND, not detected

TABLE 7

HbF Synthesis in K562 Cells

Concentration ( $\mu$ M)	AB*	AmBOMB*	POMB*	BuBOMB*
10	0.96	15	15	27.5
50	0.95	24	37	30.5
100	1.8	-	39	11.5

\*HbF in  $\mu$ g/mL.TABLE 8

Percentage Erythroid Cells Containing HbF

Treatment	Concentration ( $\mu$ M)	% HbF	% Increase
Untreated		2.08	
BuBOMB	100	2.55	122
	200	4.93	237
POMB	100	2.7	129
	200	2.36	113
BuBuOMB	100	2.52	121
	200	2.83	136

## EXAMPLE 15

Altering HER-2/neu and ICAM Gene Expression

5 Increased expression of HER-2/neu is associated with  
poor prognosis in breast cancer patients so that reducing  
expression of this gene can be beneficial to cancer  
patients. In contrast, increased expression of the  
extracellular matrix protein ICAM-1 is associated with  
maturation, i.e., differentiation, of AU565 cancer cells  
10 so that increasing ICAM expression can be beneficial to  
cancer patients.

The relative levels of expression of these two genes  
was measured by fixing and using immunocyto staining with  
a monoclonal antibody (MAb) to measure the quantities of  
15 each gene product. The cultured cells were AU565, a  
breast carcinoma cell line, grown in the presence of POMB  
or AB. The primary MAb was ICAM-1 specific and the  
primary MAb for detecting HER-2/neu was N24 (from the  
Weizmann Institute). Cells were also counterstained for  
20 DNA content using a modified Feulgen method. Antibody  
binding was determined using an alkaline phosphatase  
chromogen in a biotin-streptavidin sandwich assay and  
quantitated by image analysis. The basal expression  
level of each gene was determined on AU565 cells grown at  
25 low density, in the absence of POMB or AB and was  
arbitrarily set at 1.0 to determine relative expression  
levels.

The results shown in Table 9 demonstrate that  
POMB inhibits the expression of the oncogene HER-2/neu  
30 and induces increased expression of the ICAM gene product  
relative to AB.

TABLE 9

Expression of HER-2/Neu and ICAM

60

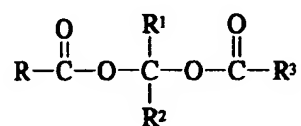
	HER-2/Neu	ICAM
Control	1.0	0
POMB	0.36	1.2
AB	1	0

5

\* Relative concentrations

## WE CLAIM:

1. A method of treating, preventing or ameliorating cancer or other proliferative disorder in a subject which comprises administering an amount of a compound effective to treat, prevent or ameliorate said cancer or said disorder, wherein said compound is represented by the formula:



wherein

R is C<sub>2</sub> to C<sub>10</sub> alkyl, alkenyl or alkynyl, any of which can be optionally substituted with aryl, heteroaryl, halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro, trifluoromethyl, acylamino or carbamoyl;

R<sup>1</sup> and R<sup>2</sup> are independently H, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>2</sub>-C<sub>10</sub> alkenyl, or C<sub>2</sub>-C<sub>10</sub> alkynyl, any of which can be optionally substituted with halo, alkoxy, amino, trifluoromethyl, aryl or heteroaryl;

R<sup>3</sup> is

(1) a nitrogen-containing heterocycloalkyl, heterocycloalkenyl, heteroaryl group or heteroaralkyl group, wherein said heteroaryl group and said heteroaralkyl group can have up to two additional substituents;

(2) an aryl or aralkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino,

- 5           dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, alkoxycarbonylamino, formamido, guanidino, ureido, sulfamyl, and alkylsulfonamido, wherein said aryl or aralkyl group can have up to two additional substituents; or
- 10           (3) a C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl, C<sub>3</sub> to C<sub>10</sub> alkynyl or C<sub>3</sub> to C<sub>7</sub> cycloalkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, alkoxycarbonylamino, formamido, guanidino, ureido, sulfamyl, and alkylsulfonamido; or
- 15           a pharmaceutically-acceptable salt thereof;

with the provisos that R is not substituted or unsubstituted n-propyl; and that when R is t-butyl and R<sup>1</sup> and R<sup>2</sup> are both hydrogen, then R<sup>3</sup> can not be 3-aminopropyl or 3-(t-butoxycarbonylamino)propyl.

20

2. The method of Claim 1, wherein

25           R is C<sub>3</sub> to C<sub>6</sub> alkyl, alkenyl or alkynyl, any of which can be optionally substituted with aryl, heteroaryl, halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro, trifluoromethyl, acylamino or carbamoyl; and

R<sup>3</sup> is

- 30           (1) a nitrogen-containing heterocycloalkyl, heteroaryl group or heteroaralkyl group, wherein said heteroaryl group and said heteroaralkyl group can have up to two additional substituents;
- 35           (2) an aryl or aralkyl group having one nitrogen-containing substituent selected from the

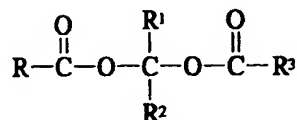


group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino and alkoxycarbonylamino, wherein said aryl or aralkyl group can have up to one additional substituent; or

(3) a C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl, C<sub>3</sub> to C<sub>10</sub> alkynyl or C<sub>3</sub> to C<sub>7</sub> cycloalkyl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino and alkoxycarbonylamino.

3. The method of Claim 2, wherein R<sup>2</sup> is H.

4. A method of treating, preventing or ameliorating cancer or other proliferative disorder in a subject which comprises administering an amount of a compound effective to treat, prevent or ameliorate said cancer or said disorder, wherein said compound is represented by the formula:



wherein

R is n-propyl which can be optionally substituted with aryl, heteroaryl, halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro, trifluoromethyl, acylamino or carbamoyl;

R<sup>1</sup> and R<sup>2</sup> are independently H, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>2</sub>-C<sub>10</sub> alkenyl, or C<sub>2</sub>-C<sub>10</sub> alkynyl, any of which can be optionally substituted with halo, alkoxy, amino, trifluoromethyl, aryl or heteroaryl;

5

R<sup>3</sup> is

(1) a nitrogen-containing heterocycloalkyl, heterocycloalkenyl, heteroaryl group or heteroaralkyl group or an alkylammonium salt thereof, wherein said heteroaryl group and said heteroaralkyl group can have up to two additional substituents;

(2) an aryl or aralkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, alkoxycarbonylamino, formamido, guanidino, ureido, sulfamyl, and alkylsulfonamido, wherein said aryl or aralkyl group can have up to two additional substituents; or

(3) a C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl, C<sub>3</sub> to C<sub>10</sub> alkynyl or C<sub>3</sub> to C<sub>7</sub> cycloalkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, alkoxycarbonylamino, formamido, guanidino, ureido, sulfamyl, and alkylsulfonamido; or

a pharmaceutically-acceptable salt thereof;

with the proviso that when R<sup>3</sup> is C<sub>1</sub> to C<sub>10</sub> alkyl, then said nitrogen-containing substituent can not be amino or alkoxycarbonylamino.

35

5. The method of Claim 4, wherein

$R^1$  and  $R^2$  are independently H, lower alkyl, lower alkenyl or lower alkynyl; and

5

$R^3$  is

(1) a nitrogen-containing heterocycloalkyl, heteroaryl group or heteroaralkyl group, wherein said heteroaryl group and said heteroaralkyl group can have up to two additional substituents;

(2) an aryl or aralkyl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, and alkoxycarbonylamino, wherein said aryl or aralkyl group can have up to one additional substituents; or

(3) a  $C_1$  to  $C_{10}$  alkyl,  $C_2$  to  $C_{10}$  alkenyl,  $C_3$  to  $C_{10}$  alkynyl or  $C_3$  to  $C_7$  cycloalkyl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino and alkoxycarbonylamino.

6. The method of Claim 4, wherein

R is n-propyl;

$R^1$  is H or lower alkyl and  $R^2$  is H; and

30

$R^3$  is

(1) a nitrogen-containing heteroaryl group, wherein said heteroaryl group can have one additional substituent;

(2) an aryl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, and wherein said aryl group  
5 can have up to two additional substituents; or

(3) a C<sub>1</sub> to C<sub>10</sub> alkyl group having one nitrogen-containing substituent selected from the group consisting of acylamino, arylcarbonylamino and alkoxy carbonylamino.

10 7. The method of Claim 6, wherein said compound is  
4-(butyramido)butyroyloxymethyl butyrate,  
4-(butyramido)benzoyloxymethyl butyrate,  
4-aminobenzoyloxymethyl butyrate,  
15 4-aminobenzoyloxymethyl butyrate hydrochloride,  
1-(4-aminobenzoyloxy)ethyl butyrate,  
3-pyridinecarboxymethyl butyrate,  
1-(3-pyridinecarboxy)ethyl butyrate, or  
(N-methylpyridinium)-3-carboxymethyl butyrate chloride.

20 8. The method of Claim 1 or 4 wherein said  
disorder is leukemia, squamous cell carcinoma, prostate  
carcinoma, breast carcinoma, colon carcinoma, pancreatic  
carcinoma, lung carcinoma, renal carcinoma, neuroblastoma  
25 or melanoma.

9. The method of Claim 1 or 4 wherein said compound  
is administered orally, parenterally, transdermally,  
transmucosally, intranasally, rectally or topically.

30 10. The method of any one of Claims 1 to 7, wherein  
said effective amount is an amount effective to inhibit  
histone deacetylase in said subject.

11. A method of differentiating or blocking proliferation of cancerous or neoplastic cells comprising administering to said cells an amount of a compound of any one of Claims 1 to 7 effective to cause  
5 differentiation of or to block proliferation of cancerous or neoplastic cells.

12. The method of Claim 11 wherein said cells are *in vivo*.  
10

13. The method of Claim 11 wherein said cells are *in vitro*.

14. The method of Claim 11 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.  
15

15. A method of increasing hemoglobin content in blood which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 7 to a subject.  
20

16. The method of Claim 15 wherein said hemoglobin is fetal hemoglobin.  
25

17. The method of Claim 15 wherein said composition is administered orally or parenterally.

18. A method of treating a blood disorder in a subject which comprises administering a therapeutically-effective amount of a compound of any one of Claims 1 to 7 to a subject.  
30

19. The method of Claim 18, wherein treating said blood disorder comprises increasing the hemoglobin content in cells of said subject.

5           20. The method of Claim 18 wherein said disorder is selected from the group consisting of thalassemias, sickle cell anemias, infectious anemias, aplastic anemias, hypoplastic and hypoproliferative anemias, sideroblastic anemias, myelophthisic anemias, antibody-  
10 mediated anemias, anemias due to chronic diseases and enzyme-deficiencies, and anemias due to blood loss, radiation therapy and chemotherapy.

15           21. The method of Claim 18 wherein said composition is administered orally or parenterally.

20           22. A method of modulating an immune response in a host which comprises administering an amount of a compound of any one of Claims 1 to 7 effective to modulate said immune response.

25           23. The method of Claim 22 wherein modulating said immune response is enhancing cytokine secretion, inhibiting or delaying apoptosis in polymorphonuclear cells, enhancing polymorphonuclear cell function by  
augmenting hematopoietic growth factor secretion, inducing expression of cell surface antigens in tumor cells, enhancing progenitor cell recovery after bone marrow transplantation.

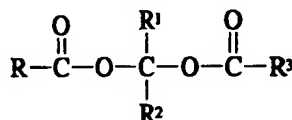
30           24. The method of Claim 22 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

25. A method of treating, preventing or ameliorating cancer or other proliferative disorder in a subject in need of such treatment which comprises administering an amount of a compound of any one of  
5 Claims 1 to 7 effective to induce cellular apoptosis of the cancer cells or of the cells of the proliferative disorder.

26. The method of Claim 25 wherein said compound is  
10 administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

27. A method for enhancing the actions of a pharmaceutical agent useful for the treatment of cancer  
15 and other proliferative disorders, comprising co-administering a therapeutically-effective amount of a compound of Formula II with a therapeutically-effective amount of said agent, wherein said agent is selected from the group consisting of a cytokine, an interleukin, an  
20 anti-cancer agent or an anti-neoplastic agent, a chemotherapeutic agent, an antibody, a conjugated antibody, an immune stimulant, an antibiotic, a hormone antagonist or a growth stimulant; and wherein said compound of Formula II is represented by the formula:

25



wherein

R is C<sub>2</sub> to C<sub>10</sub> alkyl, alkenyl or alkynyl, any of which can be optionally substituted with aryl,

heteroaryl, halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro, trifluoromethyl, acylamino or carbamoyl;

5  $R^1$  and  $R^2$  are independently H,  $C_1$ - $C_{10}$  alkyl,  $C_2$ - $C_{10}$  alkenyl, or  $C_2$ - $C_{10}$  alkynyl, any of which can be optionally substituted with halo, alkoxy, amino, trifluoromethyl, aryl or heteroaryl;

$R^3$  is

10 (1) a nitrogen-containing heterocycloalkyl, heterocycloalkenyl, heteroaryl group or heteroaralkyl group, wherein said heteroaryl group and said heteroaralkyl group can have up to two additional substituents;

15 (2) an aryl or aralkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, alkoxycarbonylamino, formamido, 20 guanidino, ureido, sulfamyl, and alkylsulfonamido, wherein said aryl or aralkyl groups can have up to two additional substituents; or

25 (3) a  $C_1$  to  $C_{10}$  alkyl,  $C_2$  to  $C_{10}$  alkenyl,  $C_3$  to  $C_{10}$  alkynyl or  $C_3$  to  $C_7$  cycloalkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, alkoxycarbonylamino, formamido, 30 guanidino, ureido, sulfamyl, and alkylsulfonamido; or

a pharmaceutically-acceptable salt thereof.

28. The method of Claim 27, wherein



R is C<sub>3</sub> to C<sub>6</sub> alkyl, alkenyl or alkynyl, any of which can be optionally substituted with aryl, heteroaryl, halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro, trifluoromethyl, acylamino or carbamoyl; and

5

R<sup>3</sup> is

(1) a nitrogen-containing heterocycloalkyl, heteroaryl group or heteroaralkyl group, wherein said heteroaryl group and said heteroaralkyl group can have up to two additional substituents;

10

(2) an aryl or aralkyl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, and alkoxycarbonylamino, wherein said aryl or aralkyl group can have up to one additional substituents; or

15

(3) a C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl, C<sub>3</sub> to C<sub>10</sub> alkynyl or C<sub>3</sub> to C<sub>7</sub> cycloalkyl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino and alkoxycarbonylamino.

20

29. The method of Claim 28, wherein R<sup>2</sup> is H.

25

30. The method of Claim 29, wherein

R is n-propyl;

30

R<sup>1</sup> is H or lower alkyl; and

R<sup>3</sup> is

(1) a nitrogen-containing heteroaryl group, wherein said heteroaryl group can have one additional substituent;

5 (2) an aryl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, and wherein said aryl group can have up to two additional substituents; or

10 (3) a C<sub>1</sub> to C<sub>10</sub> alkyl group having one nitrogen-containing substituent selected from the group consisting of acylamino, arylcarbonylamino and alkoxy carbonylamino.

15 31. The method of Claim 30, wherein said compound is 4-(butyramido)butyroyloxymethyl butyrate, 4-(butyramido)benzoyloxymethyl butyrate, 4-aminobenzoyloxymethyl butyrate, 4-aminobenzoyloxymethyl butyrate hydrochloride, 1-(4-aminobenzoyloxy)ethyl butyrate, 20 3-pyridinecarboxymethyl butyrate, 1-(3-pyridinecarboxy)ethyl butyrate, or (N-methylpyridinium)-3-carboxymethyl butyrate chloride.

25 32. The method of any one of Claims 27 to 31, wherein said antibiotic selected from the group consisting of ganciclovir, acyclovir, and famciclovir.

30 33. The method of any one of Claims 27 to 31, wherein said chemotherapeutic agent is selected from the group consisting of an alkylating agent, a purine analog, a pyrimidine analog, a vinca alkaloid, a vinca-like alkaloid, etoposide, an etoposide-like drug, a corticosteroid, a nitrosourea, an antimetabolite, a platinum-based cytotoxic drug, a hormonal antagonist, an 35 anti-androgen and an anti-estrogen.

34. The method of any one of Claims 27 to 31 wherein said cytokine is an interferon.

5 35. The method of any one of Claims 27 to 31 wherein said immune stimulant is *Corynebacterium parvum* or a sarcolectin.

10 36. The method of any one of Claims 27 to 31 wherein the chemotherapeutic agent is selected from the group consisting of tamoxifen, doxorubicin, L-asparaginase, dacarbazine, amascrine, procarbazine, hexamethylmelamine, mitosantrone and gemcitabine.

15 37. The method of any one of Claims 27 to 31 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

20 38. A method of ameliorating the effects of a cytotoxic agent which comprises administering a therapeutically-effective amount of said cytotoxic agent with a compound of any one of Claims 27 to 31 to a mammalian patient for a time and in an amount to induce growth arrest of rapidly-proliferating epithelial cells  
25 or bone marrow stem cells of said patient and thereby protecting said cells from cytotoxic effects of said agent.

30 39. The method of Claim 38, wherein said rapidly-proliferating epithelial cells are in hair follicles, gastrointestinal tract or bladder.

35 40. The method of Claim 38, wherein said rapidly-proliferating epithelial cells are hair follicle cells, or intestinal crypt cells.

41. The method of Claim 38 wherein administering is simultaneous.

5 42. The method of Claim 38 wherein administering comprises administering said agent before or after said compound.

10 43. The method of Claim 38 wherein said administration is systemic or topical.

15 44. A method of treating gastrointestinal disorders which comprises administering a therapeutically-effective amount of a compound of any one of Claims 27 to 31 to a subject.

45. The method of Claim 44 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

20 46. A method of treating cutaneous ulcers which comprises administering a therapeutically-effective amount of a compound of any one of Claims 27 to 31 to a subject.

25 47. The method of Claim 46 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

30 48. A method of inducing wound healing which comprises administering a therapeutically-effective amount of a compound of any one of Claims 27 to 31 to a subject.

49. The method of Claim 48 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intrasally, rectally or topically.

5           50. A method of enhancing recombinant gene expression which comprises treating a recombinant host cell containing an expression system for a gene product of interest with an expression-enhancing amount of a compound of any one of Claims 27 to 31, wherein said gene  
10 product is encoded by a butyric acid-responsive gene.

          51. The method of Claim 50, wherein said host cell is a mammalian cell, an insect cell, a yeast cell or a bacterial cell.  
15

          52. The method of Claim 50, wherein said gene product is a tumor suppressor gene or fetal hemoglobin.

          53. A method of treating, preventing or  
20 ameliorating symptoms in insulin-dependent patients which comprises administering an amount of a compound of any one of Claims 27 to 31 effective to enhance insulin expression.

25           54. The method of Claim 53 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

          55. A method of treating, preventing or  
30 ameliorating symptoms in cystic fibrosis patients which comprises administering an amount of a compound of any one of Claims 27 to 31 effective to enhance chloride channel expression.

56. The method of Claim 55 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

5 57. A method of inhibiting telomerase activity in cancer cells which comprises administering to said cells an amount of a compound of any one of Claims 27 to 31 effective to decrease the basal level telomerase activity in said cells and thereby inhibit malignant progression  
10 of said cells.

58. The method of Claim 57 wherein said cells are *in vivo*.

15 59. The method of Claim 58 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

20 60. The method of Claim 57 wherein said cells are *in vitro*.

61. A method of treating, preventing or ameliorating virus-associated tumors which comprises co-administering a therapeutically-effective amount of a  
25 compound of any one of Claims 27 to 31 with a therapeutically-effective amount of an antiviral agent.

62. The method of Claim 61 wherein said antiviral agent is ganciclovir, acyclovir, or famciclovir.  
30

63. The method of Claim 61 wherein said virus-associated tumor is an EBV-associated malignancy, Kaposi's sarcoma, an AIDS-related lymphoma, an hepatitis B-associated malignancy or an hepatitis C-associated  
35 malignancy.

64. The method of Claim 63 wherein said EBV-associated malignancy is nasopharyngeal carcinoma or non-Hodgkin's lymphoma.

5           65. The method of Claim 61 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

10           66. A method of modulating gene expression which comprises treating a host or host cells with an amount of a compound of any one of Claims 27 to 31 effective to enhance augment or repress expression of a gene of interest.

15           67. The method of Claim 66 wherein expression of said gene is enhanced or augmented.

20           68. The method of claim 67 wherein said gene encodes a gene product which is or acts as a repressor, a tumor suppressor, an inducer of apoptosis or an inducer of differentiation.

25           69. The method of Claim 68 wherein said host is a cancer patient and said gene is a tumor suppressor gene.

            70. The method of Claim 69 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

30           71. The method of Claim 66 wherein expression of said gene is repressed.

35           72. The method of Claim 71 wherein said gene encodes a gene product which is or acts as an oncogene or an inhibitor of apoptosis.

73. The method of Claim 72 wherein said gene is bcl-2.

5 74. The method of Claim 66 wherein said host cells are undergoing ex vivo gene therapy.

75. The method of Claim 66 wherein said host is a patient receiving in vivo gene therapy.

10 76. The method of Claim 75 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.

15 77. A method of inducing tolerance to an antigen which comprises administering a therapeutically-effective amount of compound of any one of Claims 27 to 31.

20 78. The method of Claim 77 wherein said antigen is a self-antigen.

79. The method of Claim 78 wherein said self-antigen is associated with an autoimmune disease.

25 80. The method of Claim 79 wherein said autoimmune disease is systemic lupus erythromatosis, rheumatoid arthritis, multiple sclerosis, myasthenia gravis or diabetes.

30 81. The method of Claim 77 wherein said antigen is present on a transplanted organ or cells.

82. The method of Claim 77 wherein said compound is administered orally, parenterally, transdermally, transmucosally, intranasally, rectally or topically.



83. A method for treating, preventing, or ameliorating protozoan infection in a subject which comprises administering to said subject an effective amount of a compound of any one of Claims 27 to 31.

5

84. The method of Claim 83, wherein said protozoan infection is malaria, cryptosporidiosis, toxoplasmosis, or coccidiosis.

10

85. The method of Claim 83 wherein said compound is administered orally, parenterally, transmucosally, intranasally or rectally.

15

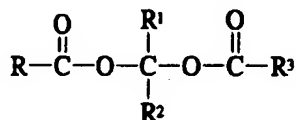
86. The method of Claim 83, wherein said effective amount is an amount of said compound effective to inhibit protozoan histone deacetylase in said subject.

20

87. A method of inhibiting histone deacetylase in cells which comprises administering an effective amount of a compound of any one of Claims 27 to 31 to said cells.

88. A compound represented by the formula:

25



wherein

R is C<sub>2</sub> to C<sub>10</sub> alkyl, alkenyl or alkynyl, any of which can be optionally substituted with aryl,

heteroaryl, halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro, trifluoromethyl, acylamino or carbamoyl;

5           R<sup>1</sup> and R<sup>2</sup> are independently H, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>2</sub>-C<sub>10</sub> alkenyl, or C<sub>2</sub>-C<sub>10</sub> alkynyl, any of which can be optionally substituted with halo, alkoxy, amino, trifluoromethyl, aryl or heteroaryl;

          R<sup>3</sup> is

10           (1) a nitrogen-containing heterocycloalkyl or heteroaryl group, wherein said heteroaryl group can have up to two additional substituents;

          (2) an aryl or aralkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, and acylamino, wherein said aryl or aralkyl group can have up to two additional substituents; or

20           (3) a C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl, or C<sub>5</sub> to C<sub>7</sub> cycloalkyl group having one or two nitrogen-containing substituents selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino and ureido; or

25           a pharmaceutically-acceptable salt thereof;

          with the provisos that R is not substituted or unsubstituted n-propyl; that when R is t-butyl and R<sup>1</sup> and R<sup>2</sup> are both hydrogen, then R<sup>3</sup> can not be 3-aminopropyl or 3-(t-butoxycarbonylamino)propyl; that when R is isopropyl and R<sup>1</sup> and R<sup>2</sup> are both hydrogen, then R<sup>3</sup> can not be 2-amino-6-chlorophenyl or 8-(3,7-dichloroquinoline); and that when R is isopropyl, R<sup>1</sup> is hydrogen, and R<sup>2</sup> is methyl, then R<sup>3</sup> can not be 4-(methylamino)cyclohexane.

30

35

89. The compound of Claim 88, wherein

R is C<sub>3</sub> to C<sub>6</sub> alkyl, alkenyl or alkynyl, any of which can be optionally substituted with aryl, heteroaryl, halo, alkyl, alkoxy, hydroxy, amino, cyano, nitro, trifluoromethyl, acylamino or carbamoyl; and

R<sup>3</sup> is

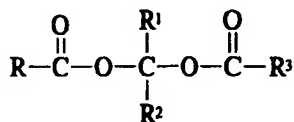
(1) a nitrogen-containing heterocycloalkyl or heteroaryl group, wherein said heteroaryl group can have up to two additional substituents;

(2) an aryl or aralkyl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino, and alkoxycarbonylamino, wherein said aryl or aralkyl group can have up to one additional substituent; or

(3) a C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl, C<sub>3</sub> to C<sub>10</sub> alkynyl or C<sub>3</sub> to C<sub>7</sub> cycloalkyl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, acylamino, arylcarbonylamino and alkoxycarbonylamino.

90. The compound of Claim 89, wherein R<sup>2</sup> is H.

91. A compound represented by the formula:



wherein

R is n-propyl which can be optionally substituted  
with aryl, heteroaryl, halo, alkyl, alkoxy, amino,  
5 hydroxy, cyano, nitro, trifluoromethyl, acylamino or  
carbamoyl;

R<sup>1</sup> and R<sup>2</sup> are independently H, C<sub>1</sub>-C<sub>10</sub> alkyl, C<sub>2</sub>-C<sub>10</sub>  
alkenyl, or C<sub>2</sub>-C<sub>10</sub> alkynyl, any of which can be optionally  
10 substituted with halo, alkoxy, amino, trifluoromethyl,  
aryl or heteroaryl;

R<sup>3</sup> is

(1) a nitrogen-containing heterocycloalkyl,  
15 heterocycloalkenyl, heteroaryl group or  
heteroaralkyl group or an alkylammonium salt  
thereof, wherein said heteroaryl group and said  
heteroaralkyl group can have up to two additional  
substituents;

20 (2) an aryl or aralkyl group having one or two  
nitrogen-containing substituents selected from the  
group consisting of amino, monoalkylamino,  
dialkylamino, trialkylammonium salts, acylamino,  
arylcarbonylamino, alkoxycarbonylamino, formamido,  
25 guanidino, ureido, sulfamyl, and alkylsulfonamido,  
wherein said aryl or aralkyl group can have up to  
two additional substituents; or

(3) a C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl, C<sub>3</sub> to C<sub>10</sub>  
alkynyl or C<sub>3</sub> to C<sub>7</sub> cycloalkyl group having one or  
30 two nitrogen-containing substituents selected from  
the group consisting of amino, monoalkylamino,  
dialkylamino, trialkylammonium salts, acylamino,  
arylcarbonylamino, alkoxycarbonylamino, formamido,  
guanidino, ureido, sulfamyl, and alkylsulfonamido;  
35 or

a pharmaceutically-acceptable salt thereof;

with the proviso that when R<sup>3</sup> is C<sub>1</sub> to C<sub>10</sub> alkyl,  
then said nitrogen-containing substituent can not be  
5 . amino.

92. The compound of Claim 91, wherein

R<sup>1</sup> and R<sup>2</sup> are independently H, lower alkyl, lower  
10 alkenyl or lower alkynyl; and

R<sup>3</sup> is

(1) a nitrogen-containing heterocycloalkyl or  
heteroaryl group, wherein said heteroaryl group can  
15 have up to two additional substituents;

(2) an aryl or aralkyl group having one  
nitrogen-containing substituent selected from the  
group consisting of amino, monoalkylamino,  
dialkylamino, trialkylammonium salts, acylamino,  
20 arylcarbonylamino, and alkoxycarbonylamino, wherein  
said aryl or aralkyl group can have up to one  
additional substituent; or

(3) a C<sub>1</sub> to C<sub>10</sub> alkyl, C<sub>2</sub> to C<sub>10</sub> alkenyl, C<sub>3</sub> to C<sub>10</sub>  
alkynyl or C<sub>3</sub> to C<sub>7</sub> cycloalkyl group having one  
25 nitrogen-containing substituent selected from the  
group consisting of amino, monoalkylamino,  
dialkylamino, trialkylammonium salts, acylamino,  
arylcarbonylamino and alkoxycarbonylamino.

30 93. The compound of Claim 91, wherein

R is n-propyl;

R<sup>1</sup> is H or lower alkyl and R<sup>2</sup> is H; and  
35

R<sup>3</sup> is

(1) a nitrogen-containing heteroaryl group, wherein said heteroaryl group can one additional substituent;

5 (2) an aryl group having one nitrogen-containing substituent selected from the group consisting of amino, monoalkylamino, dialkylamino, trialkylammonium salts, and wherein said aryl group can have up to two additional substituents; or

10 (3) a C<sub>1</sub> to C<sub>10</sub> alkyl group having one nitrogen-containing substituent selected from the group consisting of acylamino, arylcarbonylamino and alkoxycarbonylamino.

15 94. The compound of method of Claim 93, wherein said compound is

4-(butyramido)butyroyloxymethyl butyrate,

4-(butyramido)benzoyloxymethyl butyrate,

4-aminobenzoyloxymethyl butyrate,

20 4-aminobenzoyloxymethyl butyrate hydrochloride,

1-(4-aminobenzoyloxy)ethyl butyrate,

3-pyridinecarboxymethyl butyrate,

1-(3-pyridinecarboxy)ethyl butyrate, or

(N-methylpyridinium)-3-carboxymethyl butyrate chloride.

25

95. A pharmaceutical composition comprising a therapeutically-effective amount of a compound of any one of Claims 88 to 94 and a pharmaceutically-effective carrier or diluent.

30

96. A pharmaceutical composition comprising a therapeutically-effective amount of a compound of any one of Claims 88 to 94 with a therapeutically-effective amount of a pharmaceutical agent, wherein said agent is  
35 selected from the group consisting of a cytokine, an

interleukin, an anti-cancer agent or anti-neoplastic agent, a chemotherapeutic agent, an antibody, a conjugated antibody, an immune stimulant, an antibiotic, a hormone antagonist or a growth stimulant.

5

97. The composition of Claim 96 wherein said pharmaceutical agent comprises a cytotoxic agent.

98. The composition of Claim 96 wherein said  
10 antibiotic is an antiviral nucleoside antibiotic selected from the group consisting of ganciclovir, acyclovir, and famciclovir.

99. The composition of Claim 96 wherein said  
15 antibiotic is ganciclovir.

100. The composition of Claim 96 wherein said chemotherapeutic agent is selected from the group consisting of alkylating agents, purine and pyrimidine  
20 analogs, vinca and vinca-like alkaloids, etopsides and etopside-like drugs, corticosteroids, nitrosoureas, antimetabolites, platinum based cytotoxic drugs, hormonal antagonists, anti-androgens and antiestrogens.

25 101. The composition of Claim 96 wherein said cytokine is an interferon.

102. The composition of Claim 96 wherein said immune stimulant is *Corynebacterium parvum* or a  
30 sarcolectin.

103. The method of Claim 96, wherein the chemotherapeutic agent is selected from the group consisting of tamoxifen, doxorubicin, L-asparaginase,

dacarbazine, amsacrine, procarbazine, hexamethylmelamine, mitoxantrone and gemcitabine.



1/7

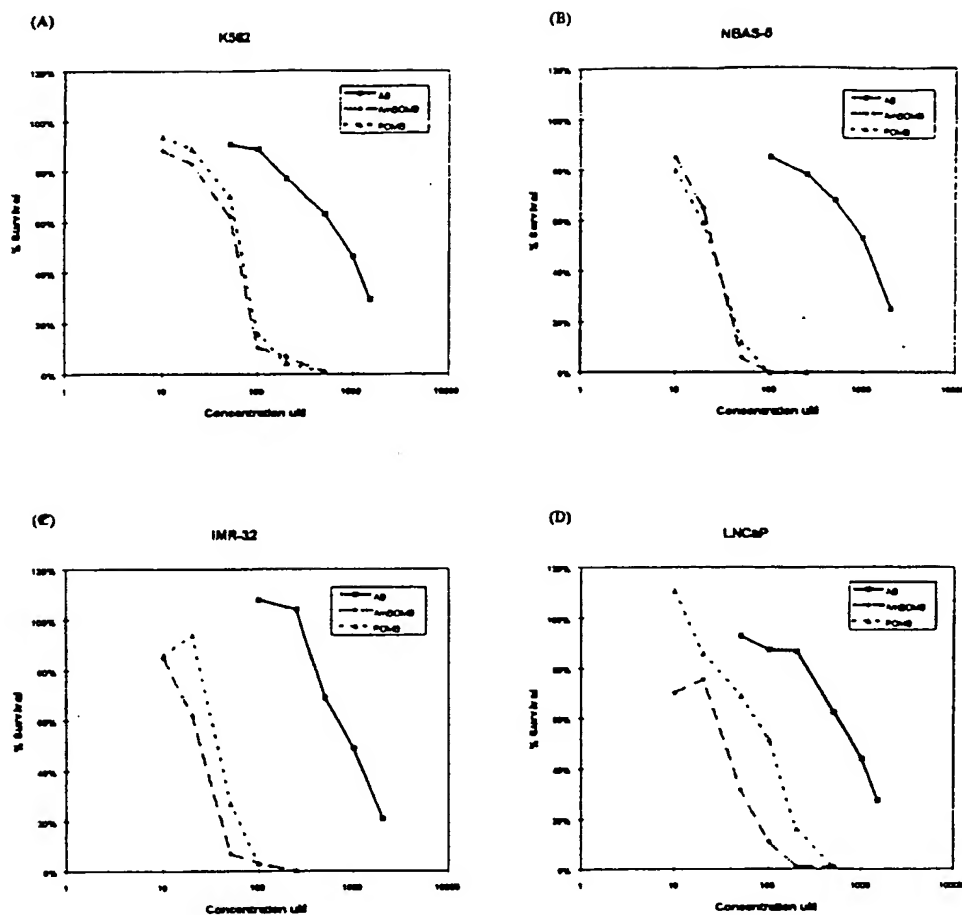


Fig. 1

2/7

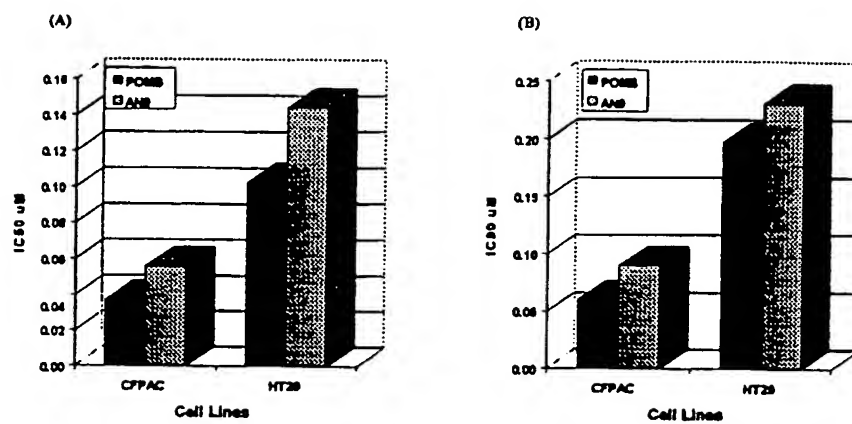


Fig. 2

3/7

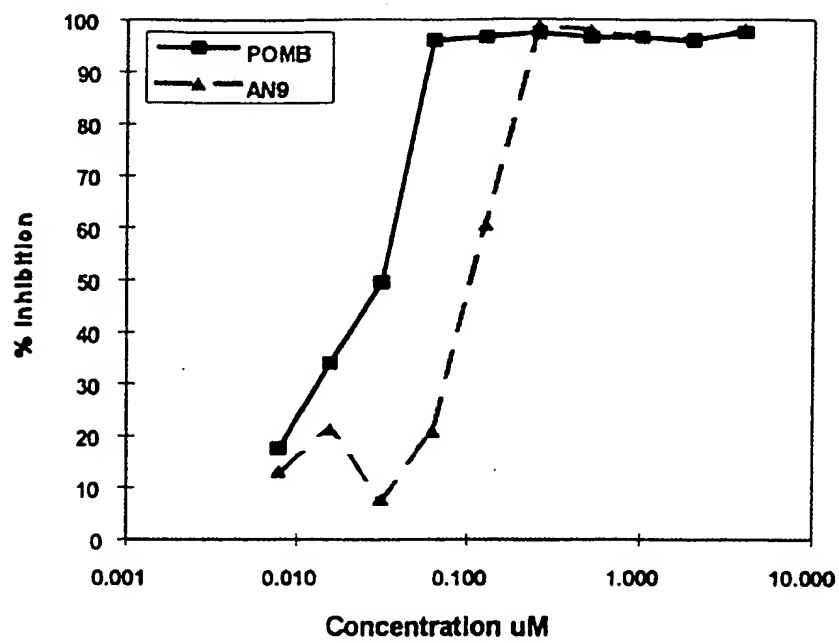


Fig. 3

4/7

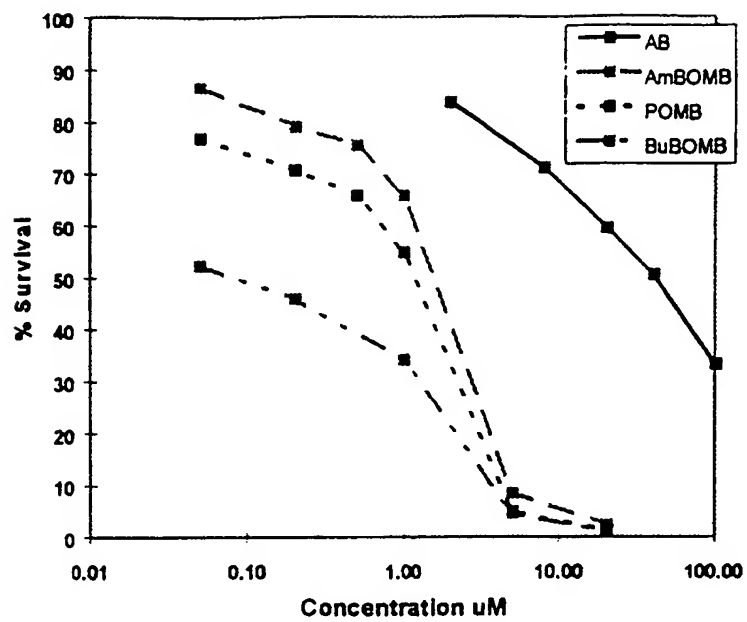


Fig. 4

5/7

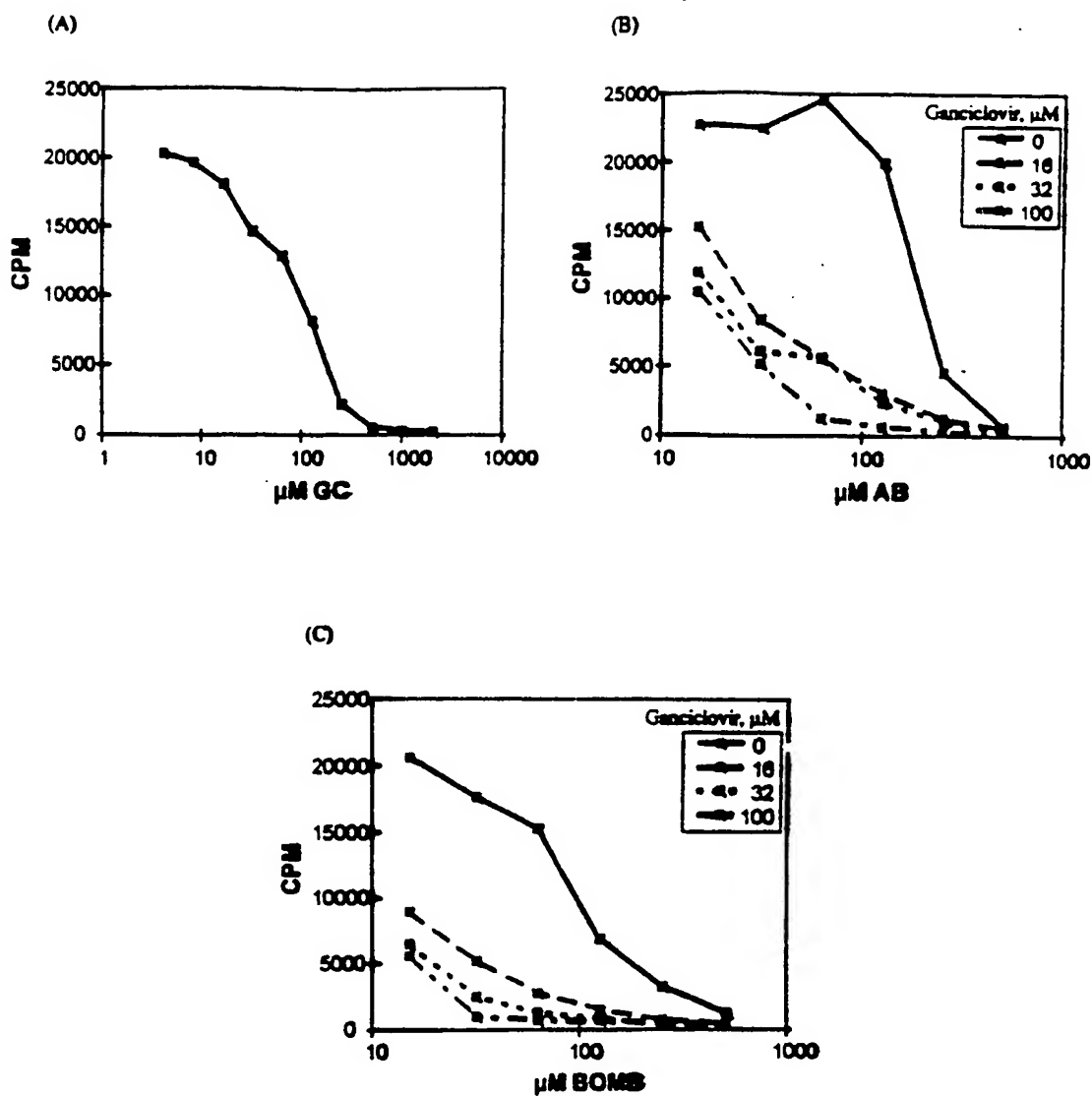


Figure 5

6/7

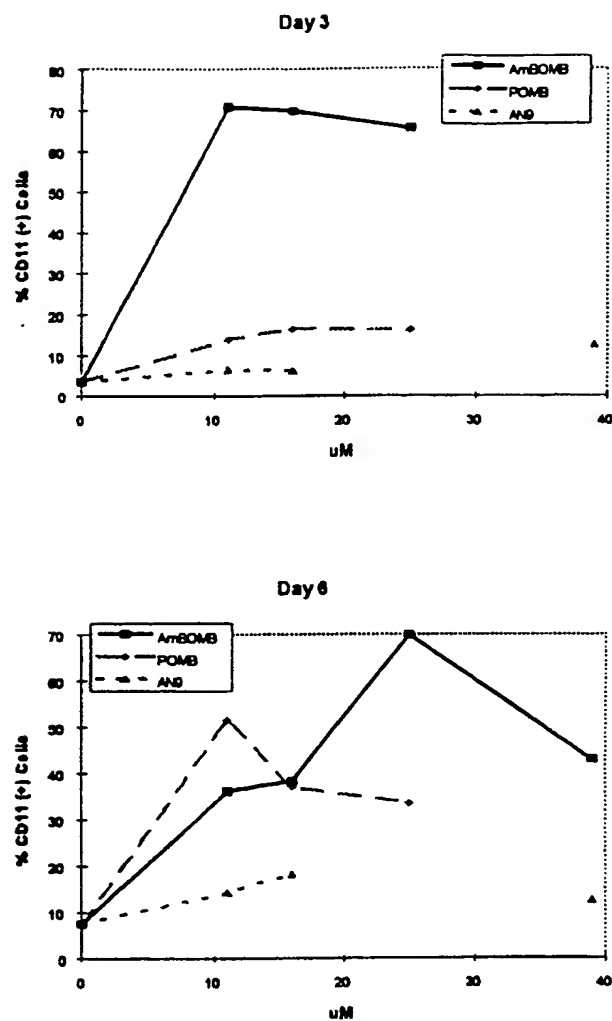


Fig. 6

7/7

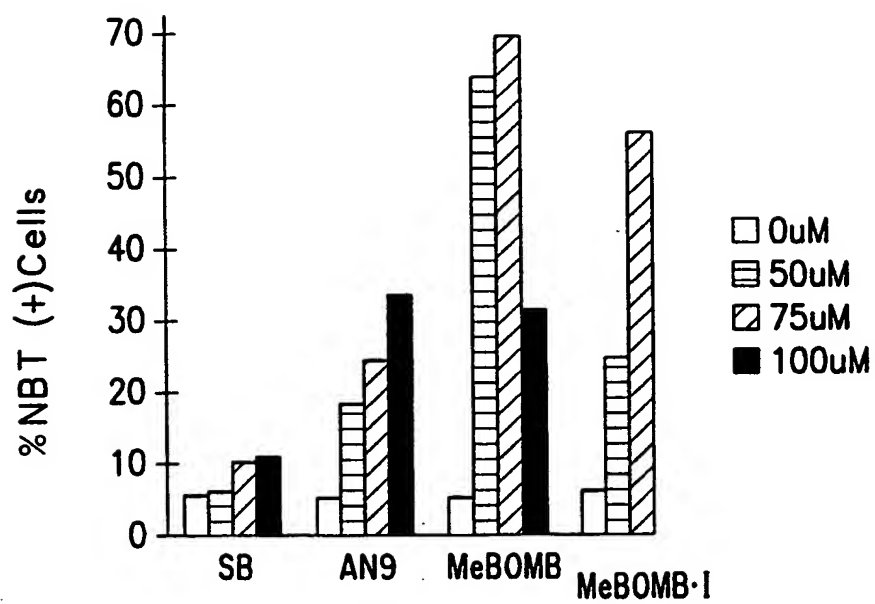


FIG. 7

## INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US98/04763

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(6) :AOIN 37/10, 55/02; CO7C 229/00; CO7D 211/78 US CL :514/533, 188; 546/318, 326; 560/50 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/533, 188; 546/318, 326; 560/50 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) CAPLUS on STN		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	US 5,710,176 A (REPHAELI et al.) 20 January 1998, entire document.	1-103
Y,P	Database CAPLUS on STN, AN 1998:55520, REPHAELI et al., 'Retinoyloxy (substituted) alkylene butyrates useful for the treatment of cancer and other proliferative diseases.' abstract of WO 98/001217 A1, 08 January 1998.	1-103
Y, E	Database CAPLUS on STN, AN 1998:175902, PURCHASE et al., 'Preparation of biphenylbutyric acids and their derivatives as inhibitors of matrix metalloproteinases.' abstract of WO 98/09940 A1, 12 March 1998.	1-103
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance "B" earlier document published on or after the international filing date "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "O" document referring to an oral disclosure, use, exhibition or other means "P" document published prior to the international filing date but later than the priority date claimed "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art "A" document member of the same patent family		
Date of the actual completion of the international search 28 APRIL 1998		Date of mailing of the international search report 19 AUG 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer <i>Brian J. Davis</i> BRIAN J. DAVIS Telephone No. (703) 308-2351